medela

Research review
Human milk safety and infection control

The NICU must ensure that the anti-infective
and nutritional properties of human milk are
provided to the infant in a risk-adverse manner.
This review highlights the evidence underpinning
safe collection, storage and handling practices
of human milk required to avoid contamination
and errors.

Medela: Comprehensive solutions for human milk and breastfeeding

For more than 50 years Medela has strived to enhance mother and baby health through the life-giving benefits of breastmilk. During this time, the company has focussed on understanding mothers' needs and infants' behaviour. The health of both mothers and their infants during the precious breastfeeding period is at the centre of all activities. Medela continues to support exploratory research into human milk and breastfeeding, and incorporates the outcomes into innovative breastfeeding solutions.

Through new discoveries surrounding the components of human milk, the anatomy of the lactating breast and how the infant removes milk from the breast, Medela has developed a set of solutions to support Neonatal Intensive Care Units (NICUs) in providing human milk and improving breastfeeding.

Medela understands the challenges of providing human milk in the NICU. There are challenges from the mother's side to reach an adequate milk supply and from the infant's side to ingest the milk; plus there are issues of hygiene and logistics when meeting these challenges. The portfolio Medela offers is directed towards obtaining human milk, promoting human milk feeding, and supporting all infants in achieving breastfeeding as early as possible.

Medela aims to provide the most recent, evidence-based knowledge to support breastfeeding and human milk use in the NICU. The goal of the innovative, research-based products, together with the educational materials, is to overcome the difficulties associated with human milk provision in the NICU.

Scientific research
Medela strives for excellence in scientific research – an attitude that has enabled the company to develop advanced breastpump and breastmilk feeding technologies. Medela works with experienced medical professionals and seeks collaboration with universities, hospitals and research institutions worldwide.

Products
Helping mothers to express milk is Medela's core competency. This includes careful and hygienic collecting of breastmilk in BPA-free containers. Easy solutions for labelling, storing, transporting, warming and thawing – all help to safely manage precious human milk. And for human milk to reach the infant, Medela has developed a range of innovative products for different feeding situations.

Education
Within Medela, research and education are closely linked. Medela connects clinicians and educators in ways that lead to professional growth, exchange of knowledge and interaction with the broader scientific community.

To put available solutions, their functionality and their interaction into the context of the overall hospital processes and evidence-based decision making, Medela has developed a series of research reviews. These reviews are available for NICU processes in which human milk and breastfeeding play a significant role. Examples are the progression of infant feeding abilities, as well as human milk logistics.

Human milk safety and infection control

Abstract

Human milk reduces the risk and severity of debilitating morbidities in preterm infants. However, retaining the integrity and safety of expressed milk is a challenging goal for the NICU. Due to the unique composition of human milk, a complex set of issues arises when collecting, storing and preparing milk for feeding. Since human milk can contain a range of commensal and potential pathogenic bacteria and viruses, some unsafe for high-risk infants, it is essential that the human milk pathway is optimised for safety and infection control. Consideration of evidence-based handling practices is crucial to ensure milk is safe for the preterm infant and remains in the nutritious and protective format of fresh milk at the breast.

Table of contents

Introduction

Internationally, breastfeeding is unanimously recommended as the sole source of nutrition for the first six months of life [1–3]. Breastfeeding provides optimal nutrition and immunological protection to the infant [4], as well as enhancement of the mother-infant pair immediately after term birth [5]. However after preterm birth, both mothers and infants may initially experience difficulties breastfeeding. Mothers need to initiate, build and maintain an adequate milk supply at an earlier developmental stage, while preterm infants often have difficulty oral feeding and are unable to breastfeed until later into the NICU (Neonatal Intensive Care Unit) stay. Therefore, preterm infants must often rely initially on expressed milk from their mothers.

The provision of expressed human milk for preterm infants is especially important in the first months of life [6]. Human milk reduces the risk and severity of debilitating morbidities in premature infants in a dose-response manner, with higher quantities of human milk leading to the greatest protection [6, 7]. However, by the time human milk is ready to be fed to the preterm infant, it has gone through a series of processes and handling steps that may reduce milk quality. Each step from the initial expression and collection, to the storage, fortification, thawing and warming of milk, can alter the integrity of human milk, expose milk to bacteria and pathogens, and potentially place the preterm infant at risk of infection. Evidence-based practices that minimise the risk of infection and maximise the quality of human milk, in both an adequate volume and integrity, are therefore essential.

This review aims to provide the NICU professional with an in-depth understanding of the latest research encompassing the health and economic benefits of human milk; how mothers can hygienically provide human milk for their infants; and the issues that the NICU faces when it comes to safe handling of human milk. Evidenced-based practices that aim to ensure that milk is of optimal integrity and minimal risk to the infant are discussed with the overall aim of maximising the use of human milk in the NICU.

Benefits of human milk for the preterm infant

Premature infants who receive human milk during their stay in the NICU have a reduced risk of necrotising enterocolitis (NEC), chronic lung disease, retinopathy of prematurity, sepsis, nosocomial infection, cognitive and neurological impairments, sudden infant death syndrome, and rehospitalisation after NICU discharge [8–17]. The powerful benefits of human milk are such that all NICU infants should receive it [2].

The positive impact of human milk appears to be linked to precise exposure in the early post-birth period, during which the exclusive use of human milk and the avoidance of commercial formula are most vital [6]. This is particularly important for hospitalised and preterm infants [2]. Preterm infants are born with immature anatomic and physiologic systems that depend on optimal nutrition for normal growth and development. Underdeveloped gastrointestinal, respiratory, neurological and immune systems result in susceptibility to the harmful effects of infection and inflammatory processes seen in the NICU. Human milk can mitigate or significantly reduce these vulnerabilities in preterm infants, thus contributing to infection control [9, 14, 16–23].

Protective components of human milk

Human milk provides the necessary components for optimal growth and development of the healthy term infant. These include the essential macronutrients (fats, carbohydrates and proteins); micronutrients (vitamins and minerals); and developmental factors (long chain polyunsaturated fatty acids (LCPUFA), growth factors and cytokines). Human milk also provides protection from infection *via* its anti-infective and immunologic components [24, 25] (Table 1).

Multi-functional macrophages and free fatty acids in human milk, as well as proteins including sIgA, lactoferrin and lysozyme, act as protective agents, which are especially important for the preterm infant [24]. These agents work together to inactivate, destroy or bind to specific microbes, thus preventing their attachment to mucosal surfaces [25]. Other components like maternal cells, which include live blood-derived leukocytes, live cells of the mammary epithelium, stem cells and cell fragments, provide immune protection to the infant [26, 27]. Human milk oligosaccharides also have an important immunological function acting as probiotics that promote the growth of commensal bacteria in the intestine [28]. They also act as decoys or receptor analogues to inhibit binding of pathogens – including rotaviruses – to intestinal surfaces [29–32]. At the same time, human milk contains protective commensal bacteria that become part of the gut microflora and influence inflammatory and immunomodulary processes. Not only do commensal bacteria prevent overgrowth of pathogenic bacteria, they also acidify the gut, ferment lactose, and breakdown lipids and proteins [33–35].

The milk of a mother who delivers a preterm infant is different from that of a mother who delivers at term. Compared with term milk, preterm milk has higher levels of energy, lipids, proteins, nitrogen, some vitamins, and minerals. In addition, preterm milk has higher levels of immune factors, including cells, immunoglobulins, and anti-inflammatory elements [36, 37]. The composition of preterm milk is especially important for gastrointestinal and neurological development and for conferring immunological protection of preterm infants [4]. Although human milk is recommended for all preterm infants [38], the nutritional composition of preterm milk cannot completely meet the high nutrient demands

for preterm infant growth, especially in infants born very low birth-weight (<1500 g) [15, 37]. For some preterm infants human milk must therefore be fortified with protein, nutrients, vitamins and minerals to ensure optimal growth and development [39]. This adds an extra handling step in which contamination risk needs to be controlled. Despite the fact that the anti-infective properties of milk protect it from contamination, there is still a chance of it becoming a source of infection if it is not handled appropriately.

Table 1 – Immunological components of human milk. Adapted from Hanson 2007 [25].

Immunological components of human milk	Function
Antibodies (especially sIgA)	The sIgA antibodies function primarily by binding microbes, preventing them from reaching the mucosal membranes, such as in the respiratory and gastrointestinal tracts [40].
Lactoferrin	The anti-bacterial activity of lactoferrin originates from its iron-binding properties, which deprive bacteria of an element necessary for their growth [41].
α-Lactalbumin	α-Lactalbumin is a major milk protein, but little is known about its functions. It has been found to have anti-tumor effects [42].
Oligosaccharides	Some of these glycans can act as prebiotic agents, selectively stimulating the growth of beneficial bacteria in the intestine. However, an even more important role is inhibiting pathogens from adhering to their target receptors on the mucosal surface of the host gastrointestinal tract [28].
Anti-secretory factor	It seems that inducing this component in milk may reduce the risk of mastitis in the mother and diarrhoea in the infant [43, 44].
Cytokines, growth factors and other signals from mother to infant	These components may function as signals from the mother to her infant, possibly helping various organs and functions to mature [45] and enhance the anti-infective function of leukocytes [46].
Fat	After enzymatic degradation, human milk lipids provide free fatty acids that can attack certain bacteria and viruses [47].
Defensins and cathelicidin	Several anti-microbial defensins and cathelicidin anti-microbial peptides have been demonstrated in human milk [48, 49].
Lysozyme	Lysozyme is an enzyme that cleaves the cell wall and the outer membrane of a variety of microorganisms, causing lysis [50].
Lactadherin	The human milk-fat globule protein lactadherin inhibits rotavirus, which is an important pathogen that causes severe dehydrating diarrhoea in infants [51].
Leukocytes	Including lymphocytes, macrophages and neutrophils. The primary role of the neutrophils and macrophages present in milk may be to protect the mammary gland against infections [26, 27].

Economic benefits of human milk

Human milk contributes to infection control by reducing the incidence, severity and/or risk of prematurity-related morbidities in a dose-response manner, most notably during the first months of life. Recent research by Patel et al.[7] has demonstrated that the dose-response relationship between prematurity-related morbidities and average daily dose of human milk (ADDHM) in the NICU is such that with every increase of human milk by 10 mL/kg/day, there was a 19 % decrease in the odds of sepsis. Infants who received the lowest daily dose of human milk (<25 mL/kg/d ADDHM) not only had the highest risk of sepsis, but also the highest NICU costs (Figure 1). The authors calculated the hospital could have saved 20,384 USD per infant or a total of 1.2 million USD by increasing their ADDHM to 25–49 mL/kg/d in the first 28 days of life. By increasing the ADDHM to ≥50 mL/kg/d beyond the first 28 days to throughout the hospital stay, 31,514 USD per infant and 1.8 million USD in hospital savings could be made.

These cost savings with human milk feeding have been replicated with other prematurity-related morbidities. Human milk feeding has been shown to reduce the severity and direct costs of late-onset sepsis, bronchopulmonary dysplasia, NEC, and retinopathy of prematurity in the NICU. Human milk feeding was also shown to reduce indirect costs of NICU hospitalisation, in that it reduced NICU hospitalisation costs independently from its impact on the prematurity-related morbidities. While there were some costs to the NICU in providing human milk [52], including the potential costs associated with safety and infection control, the economic benefits of providing human milk significantly outweighed the relatively low costs to the mother and institution [52].
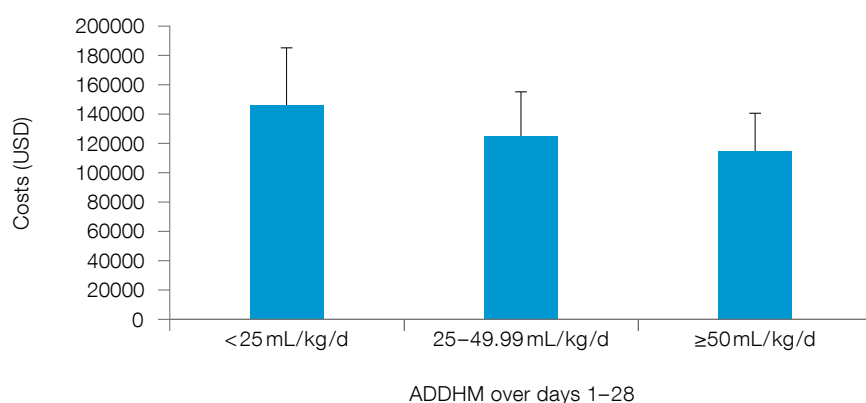

Figure 1 – Decreasing NICU costs associated with increasing dosage of human milk. Adapted from Patel et al.[7].

Safety and hygiene issues during human milk handling and feeding

Human milk is a complex, living and dynamic fluid. It is not sterile but is rather recognised as a source of transmission of commensal and pathogenic bacteria, as well as viruses. It is widely recognised that the benefits of receiving human milk, particularly own mother's milk, outweigh the challenges associated with preparing safe and hygienic milk for feeds. Careful attention to safety and hygiene during human milk handling and feeding should therefore aim to ensure that milk retains its optimal immunological and nutritional qualities for the infant [53].

Bacteria and pathogens

The components of human milk, especially live cells from the infant's mother, cannot be replaced by artificial sources. Fresh human milk, defined as milk that is either directly breastfed to the infant or newly expressed, contains live maternal cells [27] and the highest amounts of nutrients, growth factors, and many other protective components [25, 28]. Fresh human milk contains a wide range of live organisms including non-pathogenic bacteria, pathogenic bacteria, viruses, mycobacteria and fungi [54–58]. More than 700 bacterial species have been isolated in human milk [59]. These bacteria vary both in quantity and species between mothers [60]. Many of these, including intestinal bacteria, are thought to contribute to vital programming of the infant's immune system to respond appropriately to commensal and pathogenic bacteria [60]. While the quantities of bacteria in human milk vary widely, in general the majority of identified organisms are non-pathogenic normal skin flora from the mother's nipple or breast, including coagulase-negative *Staphylococcus epidermidis*, diphtheroids, and *Streptococcus viridans*. Organisms that have migrated *via* the enteromammary pathway to the breast, such as bifidobacteria or lactobacilli, which work to protect the newborn's gastrointestinal system, can also be found in human milk [61].

Human milk is also a potential vehicle for pathogenic microorganisms derived from the mother and/or the environment. Pathogenic bacteria including *Staphylococcus aureus* (MRSA), B-haemolytic streptococci*, Pseudomonas* species*, Klebsiella, Proteus* species, and enterobacteria have been identified frequently in human milk [55, 57, 61, 62]. Several outbreaks and case reports of neonatal infections have been previously linked to contaminated human milk containing *Staphylococcus aureus, Escherichia coli, Serratia* spp., *Pseudomonas* spp., *Salmonella* spp., Cytomegalovirus (CMV), and *Acinetobacter baumannii* [55, 63–65] pathogens, making safety and infection control an important issue in the NICU.

Milk can be contaminated at any point along the milk pathway. This can be during expression, collection, transport, storage and handling of milk [66]. When milk comes in contact with foreign surfaces, common skin bacterial flora and microbial species may be introduced *via* the collection apparatus [57, 67]. In particular, contaminated pumps have been identified as reservoirs for bacterial contamination, especially after being used by multiple mothers and inadequately cleaned between each use [65]. A series of case studies have shown that nearly all milk has microbial colonisation after expression by mothers of NICU infants [54, 68, 69]. Currently there are no known differences in incidence of contamination between hand expression, manual pumps, or electric pumps [42, 54, 70, 71].

Although several case studies have demonstrated that contaminated milk may be a source of infection [64, 72–76], only a few cases of infections and infection-related events have been demonstrated in preterm infants who were fed their own mother's milk (OMM). Despite it being unclear how different levels of normal bacteria affect preterm infants [54], it has been suggested that the anti-microbial properties of milk mostly protect the infant in these circumstances [77].

Nonetheless, some clinicians routinely order bacterial cultures of OMM before allowing it to be fed [78]. This practice has been more commonly observed in the US than in other countries, albeit studies demonstrating that routine initial breastmilk cultures of OMM do not predict later culture results or preterm infants' risk of infection [68, 79]. Certain situations may still warrant maternal breastmilk cultures. For example, cultures may be recommended for a NICU infant with late onset sepsis and/or recurrent Group B *Streptococcus* (GBS) or when maternal treatment for GBS takes place, particularly if the mother has mastitis, despite the incidence of GBS transmission *via* breastmilk being relatively infrequent [80–82].

Microbiological testing of fresh human milk in the NICU is a controversial topic with practices varying within countries and geographic regions [68, 83]. No universally accepted upper limits exist for bacterial colony counts in expressed human milk given to the mother's own preterm or sick infant. The criteria applied by different human milk banking organisations for pasteurised donated milk that is fed to a biologically unrelated infant have been suggested not to be as relevant to the preterm infant receiving its OMM [68, 83]. In a survey of 19 neonatal units in Belgium and Luxembourg, 47 % of units performed routine bacterial cultures of fresh milk; but among those institutions, definitions of acceptable bacterial colony counts varied significantly [68, 83]. Some units allowed $<10^5$ colony forming units (cfu) of skin commensals/mL and 10^4 cfu/mL of pathogens, while others allowed $<10^4$ cfu/mL of commensals and no pathogens. Milk containing commensal bacteria and pathogens exceeding these levels was pasteurised or, in some NICUs, discarded if colony counts were too high or any pathogens were present. In particular the discarding of fresh OMM occurred if *S. aureus* or various other pathogens were present [83]. However, only six of the 19 NICUs surveyed had access to a pasteuriser, potentially influencing the decision to discard milk. In contrast, none of the 36 neonatal units in Sweden have reported culturing or pasteurising OMM before feeding [84].

In addition to variation in bacterial limits, refrigerator storage time of fresh milk was shown to vary between 24 hours to 7 days in the NICUs studied in Belgium and Luxembourg [83]. This variation may be less common in other countries with strict storage guidelines [66, 85]. Nevertheless, variation in storage time is likely to influence the bacterial content of the milk and risk of contamination.

Due to the lack of safe upper limits with respect to bacterial and pathogen counts, it is unclear whether microbiological testing and pasteurisation of OMM are necessary. While some NICUs pasteurise OMM to reduce risks that are associated with feeding preterm infants, there is concern that infants may still be at risk due to reduced bioactive content of the milk after pasteurisation. Pasteurisation usually involves heating bottles of human milk in a water bath for 30 minutes at 62.5 °C [86]. This process is capable of a 10^5 cfu/mL [10] reduction of bacteria in human milk, however pasteurisation also affects the bioactive

nutritional and immunological components of milk [87, 88]. The important immunological proteins sIgA, lactoferrin and lysozyme are heavily affected; and retained at rates of only ~72 %, ~22 % and ~39 % after heat treatment respectively [87]. Pasteurisation also results in significant loss of white blood cells [89, 90] and has been shown to impact the resistance of milk to bacterial growth. When spiked with bacteria, the bacterial growth rate of Holder pasteurised human milk was 2-fold higher than that of raw human milk [91] (Figure 2). Consequently, there are different management processes and recommendations in place for pasteurised milk and unpasteurised milk.
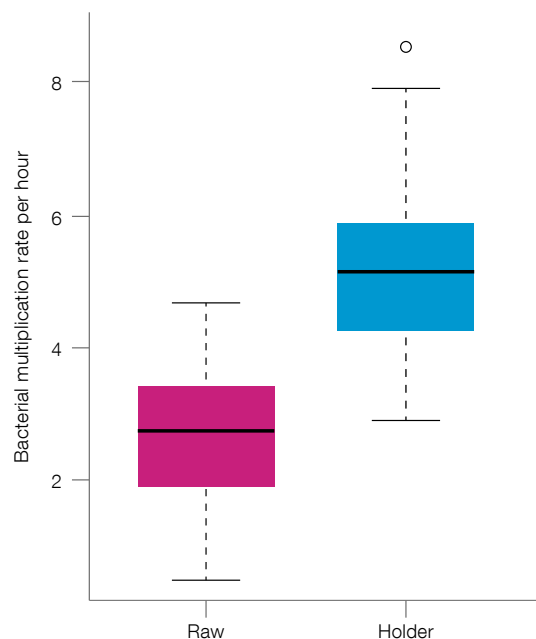
Figure 2 – Bacterial growth in Holder pasteurised human milk compared to raw human milk after being spiked with bacteria. Adapted from Christen *et al.* [91].

Viral infections and drugs

Human milk can also occasionally transmit serious viral infections to infants (Table 2). Cytomegalovirus (CMV) is a common pathogen found in the milk of women who are sero-positive for the disease. CMV is not normally a health issue for term infants who have prenatally acquired CMV antibodies *via* the placenta. However, preterm infants lacking these antibodies are at risk for CMV infection *via* breastmilk transmission [92]. The CMV transmission rate in the preterm population exposed to infected milk is also highly variable. It ranges from a rate of 6 to 55 % [92–94], depending on the presence of infectious virus in the milk, the type of viral strain, host-immune factors and the use of fresh or frozen milk [95, 96]. NICUs differ widely in practice when considering the use of fresh OMM for preterm infants when mothers are known to be CMV sero-positive. Although the risk of clinical, severe sepsis requiring additional NICU treatment due to human milk-acquired CMV is relatively uncommon, some hospitals elect to forego giving fresh OMM to preterm infants, and instead either pasteurise or freeze the milk to eliminate or reduce the risk of CMV transmission respectively [68, 83]. Other viruses, most notably HIV and human T-cell lymphotrophic virus (HTLV) type I or II, are

present in human milk and are cited as contraindications to breastfeeding or human milk feeding in most developed countries [2].

When medications and other substances such as alcohol and nicotine circulate in a mother's body, these are also contained in her breastmilk in varying concentrations. These depend on a host of factors, including maternal dose, serum levels, molecular weight, lipid solubility, pH and half-life [97]. Most reports of medication effects are based on case reports and do not usually involve the interaction of one medication with another. Therefore, while the list of medications that are contraindicated during lactation is fairly short, each maternal-infant situation must be evaluated individually when considering potential drug effects on the infant [97].

Table 2 – Infectious agents transmitted *via* breastmilk [66]

Potential infectious agents transmitted *via* human milk	Risk of infant illness
Bacteria	Unlikely to cause infection in healthy infants, especially if no clinical signs of infection are present in the mother [66].
HIV	HIV can be transmitted *via* human milk and cause illness. In developed countries, the rate of transmission is considered low because the mother is advised not to breastfeed. In developing countries, HIV transmission rates have been shown to be ~15% when infants are exclusively breastfed for six months [66, 98].
Human T cell lymphotrophic virus type 1 (HTLV-I) and 2 (HTLV-II)	HTLV-I can cause serious illness. The risk of transmission with HTLV-II is still unclear [66, 99].
Hepatitis B and C	Both Hepatitis B and C particles have been identified in human milk, but are unlikely to cause illness in the infant [100].
CMV	Transmission of CMV can occur often, however illness is mainly a concern for preterm and very low birth weight infants [68, 101, 102].
Rubella (wild type and vaccine)	Identified in human milk, but no evidence that it causes infection [66].
Herpes simplex virus types 1 and 2	Identified in human milk but transmission is unlikely, and has been mainly linked to lesions and viral shedding [103].
Varicella zoster virus (VZV)	VZV DNA has been identified in human milk. The risk of infant illness is unclear [66].

Donor milk

Donor human milk is the next best option when OMM is not acceptable in a NICU setting – as in the case of infants of mothers with HIV, HTLV-I and II; mothers taking illicit substances or contraindicated medications; or when milk is not available due to insufficient milk supply [1]. In the US, donor milk generally consists of pooled milk from various donors that has been Holder pasteurised in a human milk bank [104]. On the other hand, in Europe, guidelines from the UK specifically state not to pool milk from different donors. International standards include specific aspects of donor screening and culturing of processed milk to ensure sterility and minimal risk to preterm infants [66, 85]. While stringent guidelines for screening and pasteurising donor milk exist, the same concerns about the loss of some nutritional and immunological components after pasteurisation remain for feeding preterm infants. Donor milk banking in Norway is still based on the long tradition of using raw, unpasteurised milk for the premature infant. Only one milk bank in Norway pasteurises all donor milk and uses the milk for preterm babies less than 1500 g [105]. It is clear that OMM is preferred over donor milk, and fresh milk is preferred over frozen milk (Figure 3) and that particular care is required to minimise the risk of infection and viral transfer *via* contaminated milk.
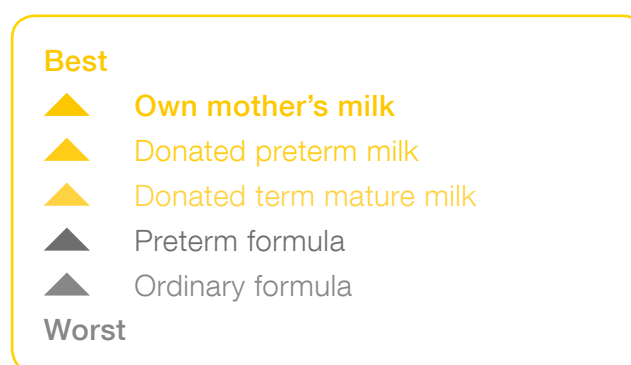
Best
▲ **Own mother's milk**
▲ Donated preterm milk
▲ Donated term mature milk
▲ Preterm formula
▲ Ordinary formula
Worst

Figure 3 – WHO recommendations for human milk feeding in the NICU. Adapted from Arnold 2002 [106].

Preventing contamination of human milk

Figure 4 – Example of hand washing covering all surfaces of the hands and fingers

Human milk should be catered to the preterm infant's needs for appropriate growth and development and provided in an optimal form, aiming for milk composition as nutritious and protective as fresh milk at the breast, and with a low risk of contamination. To achieve this, the NICU must optimise the entire human milk safety pathway starting from hygienic milk collection practices during expression to feeding the infant in the safest manner possible. Milk handling practices, especially storage, must be optimised in terms of time, temperature and labelling to minimise contamination risks and errors when handling different mothers' milk (Table 3). The principal goal when not feeding at the breast is to receive the benefits of microbiologically safe, and nutritionally and immunologically complete human milk [66].

Hand hygiene

Adherence to hand hygiene, safe milk collection practices, and cleaning and drying collection kits and the pump equipment after each use are critical steps to prevent unwanted microbiological transfer in NICUs. Hand washing is the first line of defence in reducing pathogens and bacteria [66]. Pumping mothers are recommended to wash and dry their hands thoroughly prior to pumping [66]. The evidence for washing with non-anti-bacterial soap or anti-bacterial soap for pumping mothers is unclear. Anti-microbial soaps can result in bacterial resistance [107, 108] by removing normal skin flora that serves a role in protecting skin surfaces [109] and can also have a potential effect on T cell maturation [109]. However, it has been suggested that using a sufficient volume of soap is of more concern [110]. In addition to hand cleaning, the benefit of breast cleaning beyond daily hygiene has not been shown. Breast washing with skin detergent was shown to be no more effective than water alone in reducing bacteria, and therefore only regular breast hygiene practices are recommended [58, 111].

Alcohol rubs have been implemented in hospitals and elsewhere for their convenience of use especially since no sink is required [112]. While the use of alcohol rubs is recommended in the hospital [113], there is a lack of evidence to whether there is any risk of chemical contact with the breast or milk. Therefore, no recommendations have been made whether alcohol rubs or wipes should or should not be used prior to expressing or handling either human milk or feeding equipment [66].

For healthcare workers, hand washing with either non-anti-microbial soap or anti-microbial soap and water is recommended. Hot water should be avoided because it can damage the skin. In addition, using an alcohol-based rub or an anti-microbial soap to decontaminate healthcare worker hands between patients and prior to specific patient care activities is recommended [113]. Hand washing techniques with soap and water vary as to length of washing time and amounts of soap. Healthcare workers are recommended to apply the amount of soap that is recommended by the soap manufacturer, and cover all surfaces of the hands and fingers before rinsing and drying, with the entire process of washing and drying taking 40–60 seconds [113] (Figure 4). While these studies are on healthcare personnel, they present valuable information for mothers who are practicing hand hygiene prior to breast pumping.

Hand drying presents a variety of options for consideration, including paper towels, cloth towels, and forced-air dryers. Of all these methods, drying with

a single use towel is regarded as best practice and is the most hygienic method of hand drying [114, 115], in junction with turning taps off in a way that does not recontaminate hands [113]. The literature comparing paper and cloth towels on dispenser rolls found cloth towels to be more at risk for contamination [116, 117]. Hot forced-air hand dryers appear to be safe in most environments; however, in hospital environments forced-air hand dryers may disperse bacteria, contributing to air borne contamination [117] and are therefore not recommended over paper towel drying [113].

Lastly, in terms of hand hygiene, fingernails and jewellery have been found to be factors in bacterial colonisation of hands post-cleaning [113]. The evidence-based recommendations for healthcare workers' hand hygiene include not wearing artificial fingernails or extensions, avoiding chipped nail polish, keeping nail tips to less than 6.5 mm long, and subungual areas clean [113]. Multiple studies also suggest the presence of rings can negatively impact attempts at hand cleaning. Ring wearing has been associated with 10-fold higher median skin organism colony counts, hand contamination with *Staphylococcus aureus*, gram-negative bacilli and *Candida* species [118–120]. Furthermore, the more rings an individual wears, the greater the contamination even after hand cleaning [119].

Cleaning pumps and pump sets

Breastpumps and pump sets, like all hospital equipment, are potential carriers of pathogenic microorganisms [121, 122]. Therefore, careful cleaning protocols are needed to minimise the risk of contamination of the pump used between mothers, as well as the pump sets being used repeatedly by the individual mother.

Pump sets

Pump sets usually consist of breastshields and tubing used with an electric pump. Depending on the institution and country, pump sets may require being sterile before every use. The use of sterile equipment is particularly important between mothers [123], but for many institutions, providing sterile pump sets before every use may be challenging, especially in the NICU setting with mothers pumping more than six times a day. Autoclaving or disposing after each use can prove to be both expensive and impractical. Therefore disinfection, rather than sterilisation, has become acceptable in some institutions. During their NICU stay, mothers are often given their own pump set, which can either be reusable or disposable after a day's use (approximately 8 pumping sessions). In both cases the pump sets can be disinfected rather than sterilised between uses. Discarding the pump sets after a day's use may also be preferable over autoclaving, since autoclaving is generally expensive and may risk return of incomplete sets [63, 124].

Pumping parts that come into contact with milk should be completely separated and thoroughly cleaned after use. Even in the case of no milk collection during a pumping session, pump sets should be cleaned. Methods of decontamination commonly used to clean pump sets in the NICU include chemical and steam-producing disinfection or general washing. In a survey of 25 neonatal units in the UK, chemical disinfection was the most commonly used method (56 %),

followed by autoclaving or steam-producing equipment (16 %), disposable equipment (8 %), and general washing (4 %)[63]. Each of these methods has advantages and disadvantages; decontamination by chlorine-release disinfection involves washing the pump sets prior to use, changing the solution every 24 hours, and general washing between uses[125]. General washing requires the equipment to be washed in detergent and water, rinsed, and dried *via* air exposure. With both of these methods there is a risk of cross infection if equipment is mixed up or taken by the wrong mother; and both methods are potentially dangerous to the infant if solution or detergent is not adequately rinsed off the pump set[124]. Steam-producing methods include free standing electrical steam-producing units, in which water is added at the base, as well as water-containing bags or baskets that are placed in the microwave. Steam bags or baskets are advantageous in that they can be used for individual mothers, decontamination is quick and bags are disposable. Nonetheless, both types of steam devices require care to avoid scalding[124], and both leave pump sets "wet", which may potentially enable the growth of bacteria[63, 124]. If pump sets are used repeatedly, general washing may be the simplest method of cleaning for mothers. In NICUs where most of the decontamination occurs on-site without the mothers' involvement, the optimal on-site decontamination method is still unclear[83]; more research is needed into the most practical, safe and cost-effective decontamination method[124].

For mothers pumping at home, washing is the most common method of cleaning; though it may also be commonly used in the NICU setting. After the pump sets are disassembled, they should be rinsed in cool water to remove milk residue, especially milk proteins[66]. Parts should be washed with washing-up liquid and water, either under running water or in a clean bowl or basin designated solely for this purpose. Patient specific bottle brushes can be used to clean parts, especially tight crevices[124]. Because of the high levels of bacteria in drains and sinks and on faucet handles, parts should not be placed in the sink for washing and faucet handles should be turned off with a clean paper towel[66, 113].

After washing, parts should be rinsed thoroughly and then placed on a disinfected surface for drying. Clean cloth towel drying may be acceptable, providing the towel has not been used since laundering, or air-drying is another option[66]. Once clean and dry, parts should be removed from the sink area to prevent contamination from splash back from the sink[66]. Cleaning pump sets in the dishwasher after rinsing, as an alternative to washing by hand, has also been recommended[66]. It is not necessary to clean parts such as tubing and connectors to pumps unless they are contaminated with milk, moisture or other substances. Tubing exposed to aerosol of milk or water are of concern if they become contaminated with bacteria or mould growth[66]. In these cases, the manufacturer's instructions for cleaning should be followed. Pump tubing and connectors to the pump, are not to be shared between mothers[66].

Like pump sets, bottles that are used for pumping, storing and also for feeding preterm infants must be hygienic to prevent bacterial contamination of the milk. Bottles can also be sterile and reusable after autoclaving, or clean and disposable[126]. Interestingly, no difference in colony forming units has been shown when milk is collected in sterile or clean bottles/containers[71]. Since autoclaving comes with extra costs and risks of missing bottle parts, disposable containers have been suggested to be a more attractive option in the NICU setting[83].

Table 3 – Human milk pathway and potential risks in the NICU

Human milk pathway in the NICU		Potential risk	Response
Expression:	Express at home or at the NICU	l breastpump l breastshields l storage containers l loss of volume	l appropriate hand washing and pump/pump set washing before and after pumping l consider disposable versus reusable pump sets and containers
Transport:	Transport from home or storage in the hospital	l temperature changes l mix-ups l loss of volume	l immediate labelling of all expressed milk l maintain the cool chain during transport
Storage:	Store at room, refrigerator or freezer temperature. Adding fortifiers.	l bacterial growth l loss of bioactivity of components l fortification changes	l optimal storage times should be adhered to l modify storage times depending if fresh, thawed or fortified
Prepare for feeding:	Thawing and warming	l bacterial growth l loss of bioactivity of components	l optimal temperature l consider non-water versus water devices

Pumps

In general, external surfaces of hospital breast pumps and kits, particularly those touched by mothers or staff in the process of pumping, should be disinfected between users. Both mothers and the NICU staff may be involved in cleaning hospital pumps [127]. In addition to pumps, in the hospital and at home, the surface on which cleaned pump parts are placed prior to drying should be disinfected with disinfecting solutions or wipes. If recommended by the solution's manufacturer, the surface should be rinsed with clean water after disinfection to prevent solution contamination of washed parts. Hands should also be washed after disinfecting pumps and surfaces to prevent breast or milk contact with disinfectant chemicals [66].

Tracking and transporting milk

Once milk has been expressed, how the milk is subsequently stored and fed to the infant must be considered. After expression, it should be labelled per hospital guidelines, with date and time of pumping, then refrigerated as soon as possible or frozen if it is not to be used within a short period of time. Each hospital should have policy recommendations for mothers about storage containers, conditions and times. Although mothers of healthy term infants may chill fresh milk then add it to previously frozen milk [128], no current recommendation is available for preterm infants.

Figure 5 – Example of tray used for individual storage of mother's milk in the NICU

Hospitals traditionally store mother's milk individually, immediately after each pumping session [66]. Whether mothers should store their milk individually after every pump session, or pool their milk over 24 hours has been questioned. In particular, pooling milk has been suggested as it has potential to ensure milk is more nutritionally consistent between feedings. Certainly one study [129] has shown that pooling milk over 24 hours resulted in no differences in bacterial colonisation, and a reduced variability in the caloric, protein, fat and carbohydrate content of the milk compared to individually stored milk, which varied in caloric content up to 29 %. As the nutrient content of individual pump sessions differed significantly from the 24-hour nutrient content, it was suggested that inaccurate nutrient and calorie supplementation may take place. Interestingly, pooling milk also resulted in greater maternal satisfaction than individual collection. Pooling milk may therefore provide the opportunity to tailor fortification and improve nutritional delivery to the infant [129]. Pasteurised donor milk creates a different situation and therefore one container can be used for more than one infant [66]. The use of unpasteurised donor milk is a distinct context and may have limitations for use based on circumstances of donation and hospital policy.

Pooling OMM also has advantages in terms of labelling only a single bottle compared to the requirement of labelling multiple bottles or containers after every expression. The handling and tracking of human milk, which is needed in the NICU, may be prone to errors if containers are not labelled appropriately [130]. Labelling with the patient's name, milk type, date and time of expression and volume expressed may assist in minimising milk mix-ups. Methods like storage boxes for each individual mother in a dedicated milk freezer or fridge (Figure 5) as well as bar codes, more commonly seen in milk banks, may also be advantageous [66, 131, 132].

Mothers transporting milk from home to hospital likely require instruction on how to keep milk cold during transport with freezer packs and carry bag insulation such as crumpled newspapers to remove dead air space around the milk containers and freezer packs [66]. Once expressed milk arrives at the hospital, policies should dictate practices for safe handling and administration. In the case of an infant getting another mother's milk in error, hospital policy should guide staff with information regarding disease transmission *via* human milk, screening of mothers and recipient infant for diseases, and action plans based on testing results [66].

Storage of milk in the NICU

Safe storage of milk in the NICU is essential to ensuring optimal nutrition for the infant. Storage guidelines differ based on the infant (high risk / preterm, term infant, or older) and whether the milk is fresh, frozen, thawed or fortified. The live cells in fresh milk as well as the nutrients, growth factors, and many other protective components as lactoferrin, secretory IgA and lysozyme [25], decline in potency over time with exposure to varying temperatures. At the same time, the risk of bacterial contamination and growth of pathogens in the milk increases. The effects of storage on the microbiological content, lipid composition, cellular components, anti-bacterial properties and anti-oxidant capacity have been investigated to a certain degree in human milk; however, there are still many unknowns when it comes to thawing and fortifying milk, and many recommen-

dations exist based on expert opinion. It is clear, however, that different problems arise from storage at various temperatures, along with changes with time and storage environment.

Storage containers

Human milk in the NICU should be collected and stored in a way that has minimal impact on the nutritional and immunologic composition of the milk. Milk retains most of its immunological properties in glass or hard plastic containers that do not contain polyethylene [133]; therefore hard plastic or glass are preferable for storing human milk [66, 134, 135]. Use of polyethylene containers has been associated with a 60 % drop in immunoglobulin A [133] and stainless steel containers are correlated with a decline in cell count and cell viability, when compared to polyethylene and to glass [66, 134, 135]. In addition, containers made with bisphenol A (BPA) are no longer used as infant bottles because of its adverse effects [136]. Ideal containers for milk storage for the hospitalised infant are therefore glass or food-grade hard plastic made without BPA with leak-proof lids. Clean, aseptic or sterile containers may also be acceptable, however, institutional policies may specify one over the other [66].

Room temperature storage

Since human milk contains both commensal and pathogenic bacteria, bacterial growth is a primary concern of milk storage. Nevertheless, human milk has been shown to be resistant to bacterial growth for short periods of time and under cooler temperatures. Studies of bacterial growth at room temperatures show varied results, in part, because the definition of room temperature can vary between 16–29 °C or more [137–139]. In NICUs where infants are immunocompromised, storage times for room temperature milk tend to be more conservative than those for term infants, with recommendations for immediate refrigeration of fresh milk whenever possible and a limit of up to four hours at room temperature [66].

A key study [140] assesing milk degradation at 15, 25 and 38 °C over 24 hours showed that although proteolysis and digestive enzyme changes were minimal at 15 and 25 °C after 24 hours, lipolysis occurred rapidly within a few hours of storage at 38 °C, resulting in an increase in free fatty acid concentration between 440 and 710 %. Similarly, bacterial growth, which was mainly restricted to non-pathogens, was minimal at 15 °C and remained low at 25 °C for the first 4–8 hours, but increased rapidly after 4 hours when stored at 38 °C [140]. The authors concluded that milk at 15 °C was safe for 24 hours, and at 25 °C for 4 hours [140]. More rigorous methods used to target protein activity in milk at 25 °C have since shown further reductions in β-casein over 24 hours [141, 142] and reductions in lipase within 2 hours of storage [141]. In the NICU setting, optimal storage at room temperature has been recommended to be <4 hours [66] (Table 4). Continuous feeds can therefore be safely given over a four hour period.

Refrigeration

Refrigeration at approximately 4 °C preserves the integrity of human milk longer than when it is left at room temperature [143]. The most comprehensive study assessing storage at 4 °C suggests that the maximum time fresh milk should be stored under refrigeration conditions is 96 hours (4 days) [138] (Table 4). At 96 hours fresh refrigerated milk showed no significant changes in osmolality, total and gram-negative bacterial colony counts, macronutrients and immune factors, including fat, sIgA and lactoferrin. However, the effects beyond 96 hours were not measured. In addition, refrigeration has been shown to inhibit gram-positive bacterial growth [144]. Rises in free fatty acid concentrations, and subsequent increases in acidity as a result of lipolysis, have also been consistently observed in refrigeration studies [138, 145]. Products of lipolysis are not considered to be a risk as they are associated with anti-microbial activity against bacteria, viruses and protozoa [137, 138, 145–147]. Loss of white cell counts, including macrophages and lymphocytes, as well as total proteins, have been observed at 48 hours [138]. Based on these studies, optimal storage at 4 °C has been suggested for < 4 days, especially for NICU infants if milk was freshly expressed, unfortified and not previously frozen [66]. However, practices likely vary between institutions and countries, for example some neonatal units in Belgium and Luxembourg refrigerate fresh milk up to seven days [83].

Freezing

Freezing at −20 °C, for up to 3 months has been recommended as optimal in the NICU [66]. At 3 months, vitamins A, E, and B, total protein, fat, enzymes, lactose, zinc, immunoglobulins, lysozyme, and lactoferrin are maintained, although there may be loss of vitamin C after 1 month [66]. Bacterial growth is not a significant issue for up to 6 weeks [148, 149]. The anti-bacterial capacity, however, is generally less than that of fresh milk [150, 151], due to the loss of live cells such as phagocytes. Up to 12 months at < −20 °C deep freezing is considered acceptable in the NICU [66]. Deep freezing at −80 °C may be more appropriate to maintain the bactericidal capacity of human milk, especially in NICU settings [66]. Changes in taste and smell may occur at −80 °C as lipase continues to break down fat into fatty acids [128]. In addition, studies have shown the viral load such as CMV in milk is reduced significantly after freezing but not destroyed [68, 83].

Re-freezing milk after thawing in the fridge has been shown to maintain a safe bacterial load [152]; however, milk that has been completely thawed to room temperature has been suggested to be unsafe, and should not be re-frozen [66]. There is limited evidence for appropriate storage times after thawing to room temperatures, as well as for the effect on milk quality of various transfers between containers and temperatures. However, even milk that has been frozen for several months is more beneficial than formula. On the other hand, the bioavailability and concentration of some protective components are reduced after freezing, reiterating that fresh milk is still preferred over frozen milk [66]. Refrigerated milk is considered fresh, so it should be used before milk that has been frozen [66].

Table 4 – Human milk storage guidelines for NICU infants. Adapted from HMBANA [42]

Human milk	Optimal storage time
Freshly expressed milk Room temperature: Refrigerator: Freezer:	≤ 4 hours [150] ≤ 4 days [138] ≤ 3 months. Acceptable ≤ 12 months [153–156]
Previously frozen Room temperature: Refrigerator: Freezer:	Thaw to room temp, use within ≤ 4 hours [150] Thaw to fridge, use within ≤ 24 hours Do not refreeze
Freshly expressed, fortified Room temperature: Refrigerator: Freezer:	Do not store at room temperature ≤ 24 hours [157–161] Do not freeze
Previously frozen, fortified or pasteurised Room temperature: Refrigerator: Freezer:	Do not store at room temperature ≤ 24 hours Do not refreeze
Warmed towards body temperature Room temperature: Refrigerator: Freezer:	For completion of current feed Discard Discard

Handling milk in the NICU

Preparing milk for feeding requires a series of processes, including thawing stored milk, fortification, and warming. Each process may affect the composition of milk, and risk contamination.

Thawing

Thawing and warming of human milk are two separate processes often combined into one step in clinical practice and at home. Historically, thawing milk has taken place by either leaving it at room temperature, placing it directly in the refrigerator, or placing a bottle of milk in a container of warm water. Thawing is complete when frozen milk has become liquid, is still chilled and some ice crystals are still present. The presence of ice crystals is a visible indicator the milk has not thawed beyond a certain point [66]. Thawed milk should then be refrigerated until immediately before use and not left at room temperature for more than a few hours to prevent bacterial growth [66]. Determining the exact time required for thawing can be labour intensive, as it requires constant surveillance. The use of water baths, either in milk warmers or in cups of hot water, also introduces risks of contamination *via* tap water in communal water baths, contamination from the container, or the providers' hands [66].

Figure 6 – Example of handling human milk using an aseptic technique

Although there are limited studies investigating the optimal method to thaw milk, it is well known that heating of milk during pasteurisation results in significant losses of immunological and anti-inflammatory components of milk, including sIgA, lactoferrin, and lysozyme, as well as probiotic bacteria and white blood cells. These losses are reduced when pasteurising at lower temperatures [66]. Human milk banking guidelines [66] suggest that temperatures should not exceed 37 °C when thawing milk in a water-filled container. Microwaving and hot or boiling water are not recommended since they destroy the anti-infective properties of milk [89, 90]. There is a contamination risk with all water-based methods, as water can potentially get under or inside the bottle lid and into the milk [121]. Therefore, recommendations to prevent the water touching the bottle lid have been suggested [66, 121].

Fortification

While human milk is strongly recommended for enteral feeding and all oral feeding in the NICU, whether fresh or frozen, it may require fortification to meet the high nutrient demands for preterm infant growth. Micro- and macronutrients that are ordinarily deposited during the last trimester *in utero* [39] are substantially diminished at preterm birth, and must be replaced rapidly. Fortification is therefore recommended for all infants born <1500 g, but may also be recommended for other infants [162].

If OMM is not available, or is short in supply, donor milk is often used to supplement enteral feeding [15, 37]. Donor milk is generally lower in protein content compared to OMM, and therefore requires a greater level of fortification [15, 37]. When preterm infants reach feeding volumes of approximately 100 mL/kg/day, many hospitals will fortify human milk to increase protein, calories, calcium, phosphorous and other nutrients; although this is not a consistent practice universally [15, 37]. In the US, a human-milk-based fortifier is available to those hospitals wishing to avoid bovine-based fortifiers. Research thus far suggests that a 100 % human-milk-based diet reduces the risk of medical and surgical NEC [22, 163]. If human milk is unavailable, infants are given preterm formula; however, the nutrient bioavailability is less than that in human milk [22, 164]. Overall an exclusive human milk diet, including donor milk, with human-milk-based fortifier has been shown to reduce the risk of NEC compared to preterm formula [22].

Despite its benefits, fortification has been associated with some changes in the functional value of human milk. Fortifying with bovine fortifiers has been shown to alter and interfere with the anti-bacterial actions of human milk [160, 161]. Some fortifiers may change the composition of milk, so extra care must be taken considering contamination and storage risks. Since contamination and osmolality may increase faster in fortified milk [165, 166], guidelines and manufacturer's instructions must be observed [167]. The addition of fortifiers using aseptic techniques at room temperature or cooler has been suggested not to increase osmolality levels (Figure 6) [167]. Shortened storage durations have also been recommended with fortified milk, and change when milk is fresh or frozen, previously thawed, or duration spent at room temperature [168]. These include recommendations that fortified milk should not be left at room temperature; it should be fed or refrigerated immediately and kept in the refrigerator for only up to 24 hours prior to discarding [66] (Table 4).

Warming

Milk temperature is not only important for maintaining the integrity of milk, but may also play a role in assisting the infant's ability to tolerate gavage feeds. It has been hypothesised that milk temperature can influence infant body temperature. Infant temperature has been shown to decrease when room temperature intravenous fluids are administered; it has therefore been recommended that intravenous fluids such as blood and saline are warmed towards body temperature prior to infusion [169, 170]. In many NICUs, warming of feeds is considered an important step of the milk pathway. A series of studies assessing the effect of milk warming on preterm infant stability and gastric residuals have shown mixed results. Rectal and stomach temperatures have also been shown to be lower after room temperature gavage feeds compared to body temperature feeds [169–171]; however, no differences in metabolic rates have been observed. While one study showed preterm infants axillary temperature increased up to 0.44 °C during warmed feedings, the authors found no changes in heart rate, respiratory rate or oxygen saturation with the increased temperatures [172]. Preterm infants who were gavage fed milk at cool temperatures, room temperature, and body temperature, had lower gastric residuals and improved feeding tolerances when receiving milk at body temperature (37 °C), compared to cool temperatures (10 °C); however, the type of feed was not controlled for [173]. Other studies assessing preterm infants, have not shown any differences in body temperature, gastric emptying and heart rate between cold, room and body temperature during gavage feeds [174, 175]. While term infants are able to receive milk at cool, room or warmed temperatures [128], the evidence is less clear for preterm infants.

Similar to thawing, milk can be warmed by placing it in container of warm water or by holding the bottle under running warm water, taking care to keep the bottle lid dry to avoid contamination [66]. However, regulating and attaining optimal temperatures with water-based methods is challenging. Achieving optimal temperature includes consideration of several factors, including milk volume and milk temperature at the beginning of the warming process, the size of the milk container, and water temperature after warming and at the time of feeding. At the time of feeding, wide variations in temperature (21.8 °C to 36.2 °C), and warming times (133 and 2061 seconds) have been shown, suggesting that appropriate warming duration and determining when milk is at a desired feeding temperature are often not achieved [172]. Another study measured nurses' perception of feeding temperature in comparison to the measured temperature at the time of feeding. Similar to previous studies, a large variation in milk feeding temperature was shown over 419 milk feeds, ranging between 22 °C to 46.4 °C, with an average temperature of ~31 °C at the time of feeding [176]. It was concluded that milk was delivered to the infant at an inconsistent temperature, and nurses estimation of temperature was inaccurate in comparison to the measured temperature.

The risk of using contaminated water with water-based methods is a potential challenge in the NICU. Historically, hospital tap water has been identified as a source of nosocomial infections from bacteria as well as other contaminants. In particular, *Staphylococcus* and *Klebsiella pneumoniae* have been identified in hospital tap water used to heat infant milk [177]. These bacteria were determined to be directly responsible for an outbreak of septicaemia in the hospital's NICU. More recently, in 2013 Molina-Cabrillana and associates [178] reported an outbreak of *Pseudomonas aeruginosa* infections caused by contaminated tap water in bottle warmers.

As an alternative, dry-warming devices to heat fluids that come in contact with patients have been suggested in the US [179]. The CDC (Centre of Disease Control) in their 2003 Guideline for Infection Control in Health-Care Facilities [180] suggested facilities remove sources of contaminated water whenever possible. These guidelines state that moist environments and water-based solutions can serve as reservoirs for waterborne microorganisms in hospital settings. More recently, following a tap water related *Pseudomonas* outbreak and the death of three infants in a NICU in Northern Ireland, the RQIA (Regulation and Quality Improvement Authority) issued recommendations not to warm or defrost milk by placing the container in warm tap water [181]. Some NICUs now use dry, waterless warmers, rather than water-based methods, in order to prevent potential contamination of milk. However there is limited research on the effects on milk after thawing and warming using either of these techniques.

One study has measured the changes in milk composition using a waterless warming and thawing device compared to water-based methods [182]. No differences in terms of milk integrity between waterless thawing and warming methods and water-based methods were shown. Similar changes in milk pH, bacterial colony counts and free fatty acid concentrations were shown during milk thawing and warming, using both water-based and waterless methods. However, when milk was maintained at room temperature for four hours after being thawed and warmed in the waterless device, the greatest increase in bacterial colony counts and free fatty acids was observed. Despite the bacterial content being higher after four hours of warming, it was not different from that of fresh milk before it began processing. Unfortunately, the study did not measure the effect of warming milk for four hours using the water-based method; however, it is likely that maintaining the temperature with the water-based method is both difficult and unrealistic in the NICU setting. Nonetheless, further study into the effect of warming milk for prolonged periods is warranted.

Conclusion

Evidence-based methods that maximise the quality of human milk, while minimising the risk of contamination in the NICU are required. Consideration of the entire milk pathway in the NICU is needed to do this. This begins with safe and hygienic expression practices that assure mothers' hands, all pumps and pump collection sets are clean prior to pumping. Establishing refrigeration and freezing conditions that ensure minimal loss of nutrients, growth factors, and many other protective components in milk, as well as assuring that milk is traceable, are critical to reduce infections and mix-ups.

Thawing and warming procedures should not expose milk to high temperatures or potentially contaminated water. Moreover, fortified milk needs to be handled differently to unfortified milk in order to minimise bacterial growth whilst preserving the components of human milk.

Criteria for upper limits of bacterial colony forming units when using OMM are not universally agreed upon, making the reliance on microbiological testing and subsequent pasteurisation a controversial practice. Further research is urgently required in order to understand the effect of different microorganisms and different levels of contamination on preterm infants, to ensure infants are able to receive human milk in both maximal dosage and quality.

References

1 WHO & UNICEF. Global strategy for infant and young child feeding (World Health Organization, Geneva, 2003).

2 American Academy of Pediatrics - Section on Breastfeeding. Breastfeeding and the use of human milk. Pediatrics 129, e827-e841 (2012).

3 UNICEF Facts for life (United Nations Children's Fund, New York, 2010).

4 Callen,J. & Pinelli,J. A review of the literature examining the benefits and challenges, incidence and duration, and barriers to breastfeeding in preterm infants. Adv Neonatal Care 5, 72-88 (2005).

5 Winberg,J. Mother and newborn baby: Mutual regulation of physiology and behavior - a selective review. Dev Psychobiol 47, 217-229 (2005).

6 Meier,P.P., Engstrom,J.L., Patel,J.L., Jegier,B.J., & Bruns,N.E. Improving the use of human milk during and after the NICU stay. Clin Perinatol 37, 217-245 (2010).

7 Patel,A.L. et al. Impact of early human milk on sepsis and health-care costs in very low birth weight infants. J Perinatol 33, 514-519 (2013).

8 Arslanoglu,S., Ziegler,E.E., Moro,G.E., & WAPM working group on nutrition. Donor human milk in preterm infant feeding: evidence and recommendations. J Perinat Med 38, 347-351 (2010).

9 Bisquera,J.A., Cooper,T.R., & Berseth,C.L. Impact of necrotizing enterocolitis on length of stay and hospital charges in very low birth weight infants. Pediatrics 109, 423-428 (2002).

10 Furman,L., Taylor,G., Minich,N., & Hack,M. The effect of maternal milk on neonatal morbidity of very low-birth-weight infants. Arch Pediatr Adolesc Med 157, 66-71 (2003).

11 Hylander,M.A., Strobino,D.M., & Dhanireddy,R. Human milk feedings and infection among very low birth weight infants. Pediatrics 102, E38 (1998).

12 Hylander,M.A., Strobino,D.M., Pezzullo.J.C., & Dhanireddy,R. Association of human milk feedings with a reduction in retinopathy of prematurity among very low birthweight infants. J Perinatol 21, 356-362 (2001).

13 Quigley,M.A., Henderson,G., Anthony,M.Y., & McGuire,W. Formula milk versus donor breast milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev1-41 (2007).

14 Schanler,R., Shulman,R.J., & Lau,C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. Pediatrics 103, 1150-1157 (1999).

15 Schanler,R.J., Lau,C., Hurst,N.M., & Smith,E.O. Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. Pediatrics 116, 400-406 (2005).

16 Vohr,B.R. et al. Beneficial effects of breast milk in the neonatal intensive care unit on the developmental outcome of extremely low birth weight infants at 18 months of age. Pediatrics 118, e115-e123 (2006).

17 Vohr,B.R. et al. Persistent beneficial effects of breast milk ingested in the neonatal intensive care unit on outcomes of extremely low birth weight infants at 30 months of age. Pediatrics 120, e953-e959 (2007).

18 Meier,P.P. Breastfeeding in the special care nursery. Prematures and infants with medical problems. Pediatr Clin North Am 48, 425-442 (2001).

19 Sisk,P.M., Lovelady,C.A., Gruber,K.J., Dillard,R.G., & O'Shea,T.M. Human milk consumption and full enteral feeding among infants who weigh <1250 grams. Pediatrics 121, e1528-e1533 (2008).

20 Corpeleijn,W.E. et al. Intake of own mother's milk during the first days of life is associated with decreased morbidity and mortality in very low birth weight infants during the first 60 days of life. Neonatology. 102, 276-281 (2012)

21 Meinzen-Derr,J. et al. Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death. J Perinatol 29, 57-62 (2009).

22 Sullivan,S. et al. An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. J Pediatr 156, 562-567 (2010).

23 Taylor,S.N., Basile,L.A., Ebeling,M., & Wagner,C.L. Intestinal permeability in preterm infants by feeding type: Mother's milk versus formula. Breastfeed Med 4, 11-15 (2009).

24 Newburg,D.S. & Walker,W.A. Protection of the neonate by the innate immune system of developing gut and of human milk. Pediatr Res 61, 2-8 (2007).

25 Hanson,L.A. The role of breastfeeding in the defense of the infant in Textbook of human lactation (eds. Hale,T.W. & Hartmann,P.E.) 159-192 (Hale Publishing LLP, Amarillo TX, 2007)

26 Hassiotou,F. & Geddes,D. Anatomy of the human mammary gland: Current status of knowledge. Clin Anat 26, 29-48 (2013).

27 Hassiotou,F. et al. Breastmilk is a novel source of stem cells with multilineage differentiation potential. Stem Cells 30, 2164-2174 (2012).

28 Bode,L. Human milk oligosaccharides: Every baby needs a sugar mama. Glycobiology 22, 1147-1162 (2012).

29 Garrido,D., Kim,J.H., German,J.B., Raybould,H.E., & Mills,D.A. Oligosaccharide binding proteins from Bifidobacterium longum subsp. infantis reveal a preference for host glycans. PLoS One 6, e17315 (2011).

30 Sela,D.A. et al. An infant-associated bacterial commensal utilizes breast milk sialyloligosaccharides. J Biol Chem 286, 11909-11918 (2011).

31 Wu,S., Grimm,R., German,J.B., & Lebrilla,C.B. Annotation and structural analysis of sialylated human milk oligosaccharides. J Proteome Res 10, 856-868 (2011).

32 Etzold,S. & Bode,L. Glycan-dependent viral infection in infants and the role of human milk oligosaccharides. Curr Opin Virol 7, 101-107 (2014).

33 Caicedo,R.A., Schanler,R.J., Li,N., & Neu,J. The developing intestinal ecosystem: Implications for the neonate. Pediatr Res 58, 625-628 (2005).

34 Claud,E.C. Probiotics and neonatal necrotizing enterocolitis. Anaerobe 17, 180-185 (2011).

35 Claud,E.C. & Walker,W.A. Hypothesis: Inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. FASEB J 15, 1398-1403 (2001).

36 Schanler,R.J. Evaluation of the evidence to support current recommendations to meet the needs of premature infants: The role of human milk. Am J Clin Nutr 85, 625S-628S (2007).

37 Schanler,R.J. The use of human milk for premature infants. Pediatr Clin North Am 48, 207-219 (2001).

38 Gartner,L.M. et al. Breastfeeding and the use of human milk. Pediatrics 115, 496-506 (2005).

39 Kuschel,C.A. & Harding,J.E. Multicomponent fortified human milk for promoting growth in preterm infants. Cochrane Database Syst Rev CD000343, 1-45 (2004).

40 Hanson,L.A. Immunobiology of human milk: How breastfeeding protects babies (Pharmasoft Publishing, Amarillo Texas,2004).

41 Arnold,R.R., Brewer,M., & Gauthier,J.J. Bactericidal activity of human lactoferrin: sensitivity of a variety of microorganisms. Infect Immun 28, 893-898 (1980).

42 Lönnerdal, B. & Lien,E.L. Nutritional and physiologic significance of alpha-lactalbumin in infants. Nutr Rev 61, 295-305 (2003).

43 Svensson,K., Lange,S., Lonnroth,I., Widstrom,A.M., & Hanson,L.A. Induction of anti-secretory factor in human milk may prevent mastitis. Acta Paediatr 93, 1228-1231 (2004).

44 Zaman,S., Mannan,J., Lange,S., Lonnroth,I., & Hanson,L.A. B 221, a medical food containing antisecretory factor reduces child diarrhoea: a placebo controlled trial. Acta Paediatr 96, 1655-1659 (2007).

45 Field,C.J. The immunological components of human milk and their effect on immune development in infants. J Nutr 135, 1-4 (2005).

46 King,C. & Jones,E. The Benefits of Human Millk for the Preterm Baby in Feeding and Nutrition in the Preterm Infant (eds. Jones,E. & King,C.) 1-13 (Elsevier Churchill Livingstone, Oxford, 2005).

47 Hernell,O., Ward,H., Blackberg,L., & Pereira,M.E. Killing of Giardia lamblia by human milk lipases: An effect mediated by lipolysis of milk lipids. J Infect Dis 153, 715-720 (1986).

48 Armogida,S.A., Yannaras,N.M., Melton,A.L., & Srivastava,M.D. Identification and quantification of innate immune system mediators in human breast milk. Allergy Asthma Proc 25, 297-304 (2004).

49 Murakami,M., Dorschner,R.A., Stern,L.J., Lin,K.H., & Gallo,R.L. Expression and secretion of cathelicidin antimicrobial peptides in murine mammary glands and human milk. Pediatr Res 57, 10-15 (2005).

50 Kleinman,R.E. & Walker,W.A. The enteromammary immune system: an important new concept in breast milk host defense. Dig Dis Sci 24, 876-882 (1979).

51 Newburg,D.S. et al. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. Lancet 351, 1160-1164 (1998).

52 Johnson,T.J., Patel,A.L., Bigger,H.R., Engstrom,J.L., & Meier,P.P. Economic benefits and costs of human milk feedings: A strategy to reduce the risk of prematurity-related morbidities in very-low-birth-weight infants. Adv Nutr 5, 207-212 (2014).

53 Tudehope,D.I. Human milk and the nutritional needs of preterm infants. J Pediatr 162, S17-S25 (2013).

54 Boo,N.Y., Nordiah,A.J., Alfizah,H., Nor-Rohaini,A.H., & Lim,V.K. Contamination of breast milk obtained by manual expression and breast pumps in mothers of very low birthweight infants. J Hosp Infect 49, 274-281 (2001).

55 Novak,F.R., Da Silva,A.V., Hagler,A.N., & Figueiredo,A.M. Contamination of expressed human breast milk with an epidemic multiresistant Staphylococcus aureus clone. J Med Microbiol 49, 1109-1117 (2000).

56 Carroll,L., Osman,M., Davies,D.P., & McNeish,A.S. Bacteriology of raw breast milk. Lancet 2, 1186 (1979).

57 Eidelman,A.I. & Szilagyi,G. Patterns of bacterial colonization of human milk. Obstet Gynecol 53, 550-552 (1979).

58 Thompson,N., Pickler,R.H., Munro,C., & Shotwell,J. Contamination in expressed breast milk following breast cleansing. J Hum Lact 13, 127-130 (1997).

59 Cabrera-Rubio,R. et al. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. Am J Clin Nutr 96, 544-551 (2012).

60 Jeurink,P.V. et al. Human milk: A source of more life than we imagine. Benef Microbes 4, 17-30 (2013).

61 Perez,P.F. et al. Bacterial imprinting of the neonatal immune system: Lessons from maternal cells? Pediatrics 119, e724-e732 (2007).

62 Carroll,L., Osman,M., Davies,D.P., & McNeish,A.S. Bacteriological criteria for feeding raw breast-milk to babies on neonatal units. Lancet 2, 732-733 (1979).

63 Shetty,A., Barnes,R., Adappa,R., & Doherty,C. Quality control of expressed breast milk. J Hosp Infect 62, 253-254 (2006).

64 Godambe,S., Shah,P.S., & Shah,V. Breast milk as a source of late onset neonatal sepsis. Pediatr Infect Dis J 24, 381-382 (2005).

65 Engür,D. et al. A milk pump as a source for spreading Acinetobacter baumannii in a neonatal intensive care unit. Breastfeed Med 9, 551-554 (2014).

66 Human Milk Banking Association of North America 2011 Best practice for expressing, storing and handling human milk in hospitals, homes, and child care settings (HMBANA, Fort Worth, 2011).

67 el-Mohandes,A.E., Keiser,J.F., Johnson,L.A., Refat,M., & Jackson,B.J. Aerobes isolated in fecal microflora of infants in the intensive care nursery: Relationship to human milk use and systemic sepsis. Am J Infect Control 21, 231-234 (1993).

68 Schanler,R.J. et al. Breastmilk cultures and infection in extremely premature infants. J Perinatol 31, 335-338 (2011).

69 el-Mohandes,A.E., Schatz,V., Keiser,J.F., & Jackson,B.J. Bacterial contaminants of collected and frozen human milk used in an intensive care nursery. Am J Infect Control 21, 226-230 (1993).

70 Becker,G.E., McCormick,F.M., & Renfrew,M.J. Methods of milk expression for lactating women (Review). Cochrane Database Syst Rev1-46 (2008).

71 Pittard,W.B., Geddes,K.M., Brown,S., Mintz,S., & Hulsey,T.C. Bacterial contamination of human milk: container type and method of expression. Am J Perinatol 8, 25-27 (1991).

72 Olver,W.J., Bond,D.W., Boswell,T.C., & Watkin,S.L. Neonatal group B streptococcal disease associated with infected breast milk. Arch Dis Child Fetal Neonatal Ed 83, F48-F49 (2000).

73 Byrne,P.A., Miller,C., & Justus,K. Neonatal group B streptococcal infection related to breast milk. Breastfeed Med 1, 263-270 (2006).

74 Botsford,K.B. et al. Gram-negative bacilli in human milk feedings: Quantitation and clinical consequences for premature infants. J Pediatr 109, 707-710 (1986).

75 Cooke,R.P., Devlin,J., & Robinson,M.J. Breast milk and methicillin-resistant Staphylococcus aureus. J Hosp Infect 10, 312 (1987).

76 Widger,J., O'Connell,N.H., & Stack,T. Breast milk causing neonatal sepsis and death. Clin Microbiol Infect 16, 1796-1798 (2010).

77 Goldman,A.S. Immunologic system in human milk. J Pediatr Gastroenterol Nutr 5, 343-345 (1986).

78 Ng,D.K., Lee,S.Y., Leung,L.C., Wong,S.F., & Ho,J.C. Bacteriological screening of expressed breast milk revealed a high rate of bacterial contamination in Chinese women. J Hosp Infect 58, 146-150 (2004).

79 Boer,H.R., Anido,G., & Macdonald,N. Bacterial colonization of human milk. South Med J 74, 716-718 (1981).

80 Berardi,A. et al. Group B streptococcus late-onset disease: 2003-2010. Pediatrics 131, e361-e368 (2013).

81 Davanzo,R. et al. To feed or not to feed? Case presentation and best practice guidance for human milk feeding and group B streptococcus in developed countries. J Hum Lact 29, 452-457 (2013).

82 Jawa,G., Hussain,Z., & da,S.O. Recurrent late-onset group B Streptococcus sepsis in a preterm infant acquired by expressed breastmilk transmission: a case report. Breastfeed Med 8, 134-136 (2013).

83 Cossey,V., Johansson,A.B., de,H., V, & Vanhole,C. The use of human milk in the neonatal intensive care unit: Practices in Belgium and Luxembourg. Breastfeed Med 7, 302-306 (2012).

84 Omarsdottir,S., Casper,C., Akerman,A., Polberger,S., & Vanpee,M. Breastmilk handling routines for preterm infants in Sweden: A national cross-sectional study. Breastfeed Med 3, 165-170 (2008).

85 National Institute for Health and Care Excellence. Donor milk banks: The operation of donor milk bank services. 2010. http://www.nice.org.uk/guidance/ CG93/chapter/1-Guidance (2014).

86 Balmer S.E. & Williams A.F. Guidelines for the establishment and operation of human milk banks in the UK. Arch Dis Child 73, 481-482 (1995).

87 Czank,C., Prime,D.K., Hartmann,B., Simmer,K., & Hartmann,P.E. Retention of the immunological proteins of pasteurized human milk in relation to pasteurizer design and practice. Pediatr Res 66, 374-379 (2009).

88 Czank,C., Simmer,K., & Hartmann,P.E. Simultaneous pasteurization and homogenization of human milk by combining heat and ultrasound: effect on milk quality. J Dairy Res 77, 183-189 (2010).

89 Quan,R. et al. Effects of microwave radiation on anti-infective factors in human milk. Pediatrics 89, 667-669 (1992).

90 Sigman,M., Burke,K.I., Swarner,O.W., & Shavlik,G.W. Effects of microwaving human milk: Changes in IgA content and bacterial count. J Am Diet Assoc 89, 690-692 (1989).

91 Christen,L., Lai,C.T., Hartmann,B., Hartmann,P.E., & Geddes,D.T. The effect of UV-C pasteurization on bacteriostatic properties and immunological proteins of donor human milk. PLoS One 8, e85867 (2013).

92 Capretti,M.G. et al. Very low birth weight infants born to Cytomegalovirus- seropositive mothers fed with their mother's milk: A prospective study. J Pediatr 154, 842-848 (2009).

93 Sharland,M., Khare,M., & Bedford- Russell,A. Prevention of postnatal Cytomegalovirus infection in preterm infants. Arch Dis Child Fetal Neonatal Ed 86, F140 (2002).

94 Meier,J. et al. Human Cytomegalovirus reactivation during lactation and mother-to-child transmission in preterm infants. J Clin Microbiol 43, 1318-1324 (2005).

95 Sabbaj,S. et al. Breast milk-derived antigen-specific CD8+ T cells: An extralymphoid effector memory cell population in humans. J Immunol 174, 2951-2956 (2005).

96 Pignatelli,S., Dal,M.P., Rossini,G., & Landini,M.P. Genetic polymorphisms among human Cytomegalovirus (HCMV) wild-type strains. Rev Med Virol 14, 383-410 (2004).

97 Hale,T.W. & Rowe,H.E. Medications and mothers' milk (Hale Publishing, Plano TX, 2014).

98 Read,J.S. Prevention of mother-to-child transmission of HIV through breast milk. Pediatr Infect Dis J 27, 649-650 (2008).

99 Biggar,R.J. et al. Human leukocyte antigen concordance and the transmission risk via breast-feeding of human T cell lymphotropic virus type I. J infect Dis 193, 277-282 (2006).

100 Shi,Z. et al. Breastfeeding of newborns by mothers carrying hepatitis B virus: a meta-analysis and systematic review. Arch Pediatr Adolesc Med 165, 837-846 (2011).

101 Jim,W.T. et al. Transmission of Cytomegalovirus from mothers to preterm infants by breast milk. Pediatr Infect Dis J 23, 848-851 (2004).

102 Miron,D. et al. Incidence and clinical manifestations of breast milk-acquired Cytomegalovirus infection in low birth weight infants. J Perinatol 25, 299-303 (2005).

103 Read,J.S., Cannon,M.J., Stanberry,L.R., & Schuval,S. Prevention of mother-to-child transmission of viral infections. Curr Probl Pediatr Adolesc Health Care 38, 274-297 (2008).

104 Human Milk Banking Association of North America 2011 Guidelines for establishment and operation of a donor human milk bank (HMBANA, Fort Worth, 2011).

105 Grøvslien,A.H. & Grønn,M. Donor milk banking and breastfeeding in Norway. J Hum Lact 25, 206-210 (2009).

106 Arnold,L.D. The cost-effectiveness of using banked donor milk in the neonatal intensive care unit: prevention of necrotizing enterocolitis. J Hum Lact 18, 172-177 (2002).

107 Tan,L., Nielsen,N.H., Young,D.C., & Trizna,Z. Use of antimicrobial agents in consumer products. Arch Dermatol 138, 1082-1086 (2002).

108 Aiello,A.E., Larson,E.L., & Levy,S.B. Consumer antibacterial soaps: Effective or just risky? Clin Infect Dis 45 Suppl 2, S137-S147 (2007).

109 Levy,S.B. Antibacterial household products: Cause for concern. Emerg Infect Dis 7, 512-515 (2001).

110 Larson,E.L., Eke,P.I., Wilder,M.P., & Laughon,B.E. Quantity of soap as a variable in handwashing. Infect Control 8, 371-375 (1987).

111 Costa,K.M. A comparison of colony counts of breast milk using two methods of breast cleansing. J Obstet Gynecol Neonatal Nurs 18, 231-236 (1989).

112 Kac,G. et al. Microbiological evaluation of two hand hygiene procedures achieved by healthcare workers during routine patient care: A randomized study. J Hosp Infect 60, 32-39 (2005).

113 Pittet,D., Allegranzi,B., & Boyce,J. The World Health Organization guidelines on hand hygiene in health care and their consensus recommendations. Infect Control Hosp Epidemiol 30, 611-622 (2009).

114 Harrison,W.A., Griffith,C.J., Ayers,T., & Michaels,B. Bacterial transfer and cross-contamination potential associated with paper-towel dispensing. Am J Infect Control 31, 387-391 (2003).

115 Harrison,W.A., Griffith,C.J., Michaels,B., & Ayers,T. Technique to determine contamination exposure routes and the economic efficiency of folded paper-towel dispensing. Am J Infect Control 31, 104-108 (2003).

116 Gustafson,D.R. et al. Effects of 4 hand-drying methods for removing bacteria from washed hands: A randomized trial. Mayo Clin Proc 75, 705-708 (2000).

117 Huang,C., Ma,W., & Stack,S. The hygienic efficacy of different hand-drying methods: A review of the evidence. Mayo Clin Proc 87, 791-798 (2012).

118 Salisbury,D.M., Hutfilz,P., Treen,L.M., Bollin,G.E., & Gautam,S. The effect of rings on microbial load of health care workers' hands. Am J Infect Control 25, 24-27 (1997).

119 Trick,W.E. et al. Impact of ring wearing on hand contamination and comparison of hand hygiene agents in a hospital. Clin Infect Dis 36, 1383-1390 (2003).

120 Hoffman,P.N., Cooke,E.M., McCarville,M.R., & Emmerson,A.M. Micro-organisms isolated from skin under wedding rings worn by hospital staff. Br Med J (Clin Res Ed) 290, 206-207 (1985).

121 Brown,S.L., Bright,R.A., Dwyer,D.E., & Foxman,B. Breast pump adverse events: Reports to the food and drug administration. J Hum Lact 21, 169-174 (2005).

122 Jones,B. et al. An outbreak of Serratia marcescens in two neonatal intensive care units. J Hosp Infect 46, 314-319 (2000).

123 D'Amico,C.J., DiNardo,C.A., & Krystofiak,S. Preventing contamination of breast pump kit attachments in the NICU. J Perinat Neonatal Nurs 17, 150-157 (2003).

124 Gilks,J., Price,E., Hateley,P., Gould,D., & Weaver,G. Pros, cons and potential risks of on-site decontamination methods used on neonatal units for articles indirectly associated with infant feeding, including breast pump collection kits and neonatal dummies. J Infect Prev 13, 16-23 (2012).

125 Atkinson,A. Decontamination of breast milk collection kits - a change in practice. Midwives Information Resource Services. Midwifery Digest 11, 381-383 (2001).

126 Cossey,V., Jeurissen,A., Thelissen,M.J., Vanhole,C., & Schuermans,A. Expressed breast milk on a neonatal unit: A hazard analysis and critical control points approach. Am J Infect Control 39, 832-838 (2011).

127 Meier,P.P., Engstrom,J.L., Mingolelli,S.S., Miracle,D.J., & Kiesling,S. The Rush Mothers' Milk Club: Breastfeeding interventions for mothers with very-low-birth-weight infants. J Obstet Gynecol Neonatal Nurs 33, 164-174 (2004).

128 Eglash,A. ABM clinical protocol #8: Human milk storage information for home use for full-term infants (original protocol March 2004; revision #1 March 2010). Breastfeed Med 5, 127-130 (2010).

129 Stellwagen,L.M., Vaucher,Y.E., Chan,C.S., Montminy,T.D., & Kim,J.H. Pooling expressed breastmilk to provide a consistent feeding composition for premature infants. Breastfeed Med 8, 205-209 (2013).

130 Zeilhofer,U.B., Frey,B., Zandee,J., & Bernet,V. The role of critical incident monitoring in detection and prevention of human breast milk confusions. Eur J Pediatr 168, 1277-1279 (2009).

131 Dougherty,D. & Nash,A. Bar coding from breast to baby: A comprehensive breast milk management system for the NICU. Neonatal Netw 28, 321-328 (2009).

132 Drenckpohl,D., Bowers,L., & Cooper,H. Use of the six sigma methodology to reduce incidence of breast milk administration errors in the NICU. Neonatal Netw 26, 161-166 (2007).

133 Goldblum,R. et al. Human milk Banking: I. Effects of container upon immunologic factors in mature milk. Nutr Res 1, 449-459 (1981).

134 Williamson,M.T. & Murti,P.K. Effects of storage, time, temperature, and composition of containers on biologic components of human milk. J Hum Lact 12, 31-35 (1996).

135 Hopkinson,J., Garza,C., & Asquith,M.T. Human milk storage in glass containers. J Hum Lact 6, 104-105 (1990).

136 vom Saal,F.S. & Hughes,C. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ Health Perspect 113, 926-933 (2005).

137 Igumbor,E.O., Mukura,R.D., Makandiramba,B., & Chihota,V. Storage of breast milk: Effect of temperature and storage duration on microbial growth. Cent Afr J Med 46, 247-251 (2000).

138 Slutzah,M., Codipilly,C.N., Potak,D., Clark,R.M., & Schanler,R.J. Refrigerator storage of expressed human milk in the neonatal intensive care unit. J Pediatr 156, 26-28 (2010).

139 Nwankwo,M.U., Offor,E., Okolo,A.A., & Omene,J.A. Bacterial growth in expressed breast-milk. Ann Trop Paediatr 8, 92-95 (1988).

140 Hamosh,M., Ellis,L., Pollock,D., Henderson,T., & Hamosh,P. Breastfeeding and the working mother: Effect of time and temperature of short-term storage on proteolysis, lipolysis, and bacterial growth in milk. Pediatrics 97, 492-498 (1996).

141 Molinari,C., Casadio,Y.S., Arthur,P.G., & Hartmann,P.E. The effect of storage at 25° C on proteins in human milk. Internat Diary J 21, 286-293 (2011).

142 Ferranti,P. et al. Casein proteolysis in human milk: Tracing the pattern of casein breakdown and the formation of potential bioactive peptides. J Dairy Res 71, 74-87 (2004).

143 Lawrence,R. Storage of human milk and the influence of procedures on immunological components of human milk. Acta Paediatr Suppl 88, 14-18 (1999).

144 Sosa,R. & Barness,L. Bacterial growth in refrigerated human milk. Am J Dis Child 141, 111-112 (1987).

145 Ogundele,M.O. Effects of storage on the physicochemical and antibacterial properties of human milk. Brit J Biomed Sci 59, 205 (2002).

146 Martinez-Costa,C. et al. Effects of refrigeration on the bactericidal activity of human milk: A preliminary study. J Pediatr Gastroenterol Nutr 45, 275-277 (2007).

147 Silvestre,D., Lopez,M.C., March,L., Plaza,A., & Martinez-Costa,C. Bactericidal activity of human milk: Stability during storage. Br J Biomed Sci 63, 59-62 (2006).

148 Marin,M.L. et al. Cold storage of human milk: Effect on its bacterial composition. J Pediatr Gastroenterol Nutr 49, 343-348 (2009).

149 Takci,S. et al. Effects of freezing on the bactericidal activity of human milk. J Pediatr Gastroenterol Nutr 55, 146-149 (2012).

150 Pardou,A., Serruys,E., Mascart-Lemone,F., Dramaix,M., & Vis,H.L. Human milk banking: influence of storage processes and of bacterial contamination on some milk constituents. Biol Neonate 65, 302-309 (1994).

151 Hernandez,J., Lemons,P., Lemons,J., & Todd,J. Effect of storage processes on the bacterial growth-inhibiting activity of human breast milk. Pediatrics 63, 597-601 (1979).

152 Rechtman,D.J., Lee,M.L., & Berg,H. Effect of environmental conditions on unpasteurized donor human milk. Breastfeed Med 1, 24-26 (2006).

153 Friend,B.A., Shahani,K.M., Long,C.A., & Vaughn,L.A. The effect of processing and storage on key enzymes, B vitamins, and lipids of mature human milk. I. Evaluation of fresh samples and effects of freezing and frozen storage. Pediatr Res 17, 61-64 (1983).

154 Evans,T.J., Ryley,H.C., Neale,L.M., Dodge,J.A., & Lewarne,V.M. Effect of storage and heat on antimicrobial proteins in human milk. Arch Dis Child 53, 239-241 (1978).

155 Buss,I.H., McGill,F., Darlow,B.A., & Winterbourn,C.C. Vitamin C is reduced in human milk after storage. Acta Paediatr 90, 813-815 (2001).

156 Bank,M.R., Kirksey,A., West,K., & Giacoia,G. Effect of storage time and temperature on folacin and vitamin C levels in term and preterm human milk. Am J Clin Nutr 41, 235-242 (1985).

157 Barash,J.R., Hsia,J.K., & Arnon,S.S. Presence of soil-dwelling clostridia in commercial powdered infant formulas. J Pediatr 156, 402-408 (2010).

158 WHO. Safe preparation, storage and handling of powdered infant formula guidelines (WHO Press, Geneva, 2007).

159 Chan,G.M. Effects of powdered human milk fortifiers on the antibacterial actions of human milk. J Perinatol 23, 620-623 (2003).

160 Chan,G.M., Lee,M.L., & Rechtman,D.J. Effects of a human milk-derived human milk fortifier on the antibacterial actions of human milk. Breastfeed Med 2, 205-208 (2007).

161 Santiago,M.S., Codipilly,C.N., Potak,D.C., & Schanler,R.J. Effect of human milk fortifiers on bacterial growth in human milk. J Perinatol 25, 647-649 (2005).

162 American Academy of Pediatrics - Committee on Nutrition. Nutritional needs of low-birth-weight infants. Pediatrics 75, 976-986 (1985).

163 Cristofalo,E.A. et al. Randomized trial of exclusive human milk versus preterm formula diets in extremely premature infants. J Pediatr 163, 1592-1595 (2013).

164 Lapillonne,A., O'Connor,D.L., Wang,D., & Rigo,J. Nutritional recommendations for the late-preterm infant and the preterm infant after hospital discharge. J Pediatr 162, S90-100 (2013).

165 Jocson,M.A., Mason,E.O., & Schanler,R.J. The effects of nutrient fortification and varying storage conditions on host defense properties of human milk. Pediatrics 100, 240-243 (1997).

166 Janjindamai,W. & Chotsampancharoen,T. Effect of fortification on the osmolality of human milk. J Med Assoc Thai 89, 1400-1403 (2006).

167 Fenton,T.R. & Belik,J. Routine handling of milk fed to preterm infants can significantly increase osmolality. J Pediatr Gastroenterol Nutr 35, 298-302 (2002).

168 Diehl-Jones,W., Askin,D.F., & Friel,J.K. Microlipid-induced oxidative stress in human breastmilk: In vitro effects on intestinal epithelial cells. Breastfeed Med 2, 209-218 (2007).

169 Nilsson,K. Maintenance and monitoring of body temperature in infants and children. Paediatr Anaesth 1, 13-20 (1991).

170 Knobel,R. & Holditch-Davis,D. Thermoregulation and heat loss prevention after birth and during neonatal intensive-care unit stabilisation of extremely low-birthweight infants. J Obstet Gynecol Neonatal Nurs 36, 280-287 (2007).

171 Eckburg,J.J., Bell,E.F., Rios,G.R., & Wilmoth,P.K. Effects of formula temperature on postprandial thermogenesis and body temperature of premature infants. J Pediatr 111, 588-592 (1987).

172 Dumm,M., Hamms,M., Sutton,J., & Ryan-Wenger,N. NICU breast milk warming practices and the physiological effects of breast milk feeding temperatures on preterm infants. Adv Neonatal Care 13, 279-287 (2013).

173 Gonzales,I., Durvea,E.J., Vasquez,E., & Geraghty,N. Effect of enteral feeding temperature on feeding tolerance in preterm infants. Neonatal Netw 14, 39-43 (1995).

174 Costalos,C., Ross,I., Campbell,A.G., & Sofi,M. Is it necessary to warm infants' feeds? Arch Dis Child 54, 899-901 (1979).

175 Anderson,C.A. & Berseth,C.L. Neither motor responses nor gastric emptying vary in response to formula temperature in preterm infants. Biol Neonate 70, 265-270 (1996).

176 Lawlor-Klean,P., Lefaiver,C.A., & Wiesbrock,J. Nurses' perception of milk temperature at delivery compared to actual practice in the neonatal intensive care unit. Adv Neonatal Care 13, E1-E10 (2013).

177 Büyükyavuz,B.I., Adiloglu,A.K., Onal,S., Cubukcu,S.E., & Cetin,H. Finding the sources of septicemia at a neonatal intensive care unit: Newborns and infants can be contaminated while being fed. Jap J Infect Dis 59, 213-215 (2006).

178 Molina-Cabrillana,J. et al. Outbreak of Pseudomonas aeruginosa infections in a neonatal care unit associated with feeding bottles heaters. Am J Infect Control 41, e7-e9 (2013).

179 Squier,C., Yu,V.L., & Stout,J.E. Waterborne nosocomial infections. Curr Infect Dis Rep 2, 490-496 (2000).

180 Sehulster,L. & Chinn,R.Y. Guidelines for environmental infection control in health-care facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). MMWR Recomm Rep 52, 1-42 (2003).

181 The Regulation and Quality Improvement Authority. Independent review of incidents of Pseudomonas aeruginosa infection in neonatal units in Northern Ireland - Final report (2012).

182 Handa,D. et al. Do thawing and warming affect the integrity of human milk?. J Perinatol 34, 863–866 (2014).

www.medela.com

Updated March 2015

Note: This document is not applicable for the US market.