Human Milk—Treatment and Quality of Banked Human Milk

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KEYWORDS

• Prematurity • Milk banking • Donor milk • Pasteurization • Nutrition • Infection • Breastfeeding • Milk

KEY POINTS

- Donor human milk (DHM) is beneficial for the health of preterm infants, and it is essential that human milk banks deliver a safe and high-quality DHM.
- Low temperature (62.5°C) long time (30 minutes) pasteurization offers the best compromise between microbiological safety and immunologic quality of DHM.
- Not all pasteurizers are equivalent to apply well-controlled temperature, and regular strict quality control is necessary.
- New techniques have been proposed, such as high temperature short time, high-pressure processing, or UV irradiation, which have been tested in experimental conditions.
- When devices usable in human milk banks will be available, it will be necessary to test these new methods in real conditions.

INTRODUCTION

Breast milk is a unique bioactive substance essential to the development of the newborn's immature immune and digestive systems. In preterm infants, there are specific benefits related to human milk (HM) that helps to reduce significantly the risk of digestive intolerance, necrotizing enterocolitis, late onset sepsis, bronchopulmonary dysplasia, and retinopathy of prematurity.[1–6] It has also a long-term positive impact on cognitive development and metabolism and cardiovascular health at adult age.[7] Therefore, HM nutrition is one of most cost-effective interventions that allow

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promoting childhood and adult health.[8] Mothers who delivered prematurely often experience significant difficulties in breastfeeding their infant. Furthermore, because preterm neonates are not able to breastfeed during the first weeks of life, they are at greatest risk of not receiving HM.

Breast milk from their own mother (MOM) is the first option for these newborns. When this is not possible, the next best option is donor human milk (DHM) from a human milk bank (HMB).[9,10] In 2010, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition advocated the use of HM for preterm infants as standard practice, provided it is fortified with added nutrients where necessary to meet requirements.[11] The presence of HMB is useful to help provide most preterm babies with HM. It improves the exposure to HM during hospitalization and breastfeeding rates at discharge.[12–14]

DONOR HUMAN MILK TO FEED PRETERM INFANTS

When fresh MOM is not available, preterm infants benefit from receiving DHM rather than a preterm formula.[5] For example, gastric emptying is faster and digestive tolerance is better in children fed with pasteurized breast milk than in infants fed a preterm formula.[15] In a recent randomized trial, Cossey and colleagues[16] reported no difference in either the digestive tolerance or the prevalence of necrotizing enterocolitis in preterm infants fed fresh versus pasteurized MOM, confirming previously reported results.[17] Indeed, the prevention of necrotizing enterocolitis by HM passes mainly through its effects on gut maturation,[18,19] and breast milk components that have a maturational effect are apparently not destroyed by pasteurization.[20,21]

The protective effect of HM against late onset sepsis in preterm infants is related to the presence of immunologic factors, most of which are sensitive to storage, freezing, and pasteurization. However, pasteurized milk retains the ability to inhibit bacterial growth even if it is slightly reduced when compared with fresh milk.[22] Two randomized trials showed no significant difference in the prevalence of late onset sepsis among preterm infants fed fresh or pasteurized HM.[16,23] Other nonrandomized studies report similar findings.[24,25] Then, available evidence suggests that pasteurized HM retains a part of its anti-infective properties and could be as effective as fresh milk to protect premature infants against infections.

Pasteurization has no significant effect on nutrients that are essential to support postnatal growth, such as energy, protein, or zinc contents.[26,27] However, it has been suggested that the pasteurization of milk could have a negative effect on growth of preterm infants, via reduced intestinal fat absorption related to destruction of bile salts–stimulated lipase (BSSL).[28,29] However, Andersson and colleagues[28] observed a nonsignificant reduction of fat absorption and no effect on weight gain in preterm infants fed pasteurized HM. The absence of significant effect on growth could be partly explained because the HM is not the only source of lipase and also because heat treatment of HM increases significantly the amount of free fatty acids that is known to be better absorbed in the digestive system.[30] Moreover, the observational study from Montjaux-Régis and colleagues[29] has compared infants fed unpasteurized MOM or mature donor milk. As the protein content of milk from mothers delivering prematurely is higher in than mature milk provided by donors, it is likely that these investigators identified the effect of HM composition rather than the effect of pasteurization[31] (**Fig. 1**). In a case-control study, Giuliani and colleagues[24] found no significant difference in weight gain between premature infants receiving MOM or pasteurized milk. The only randomized trial comparing the growth of premature infants receiving fresh versus pasteurized MOM reported no difference in weight

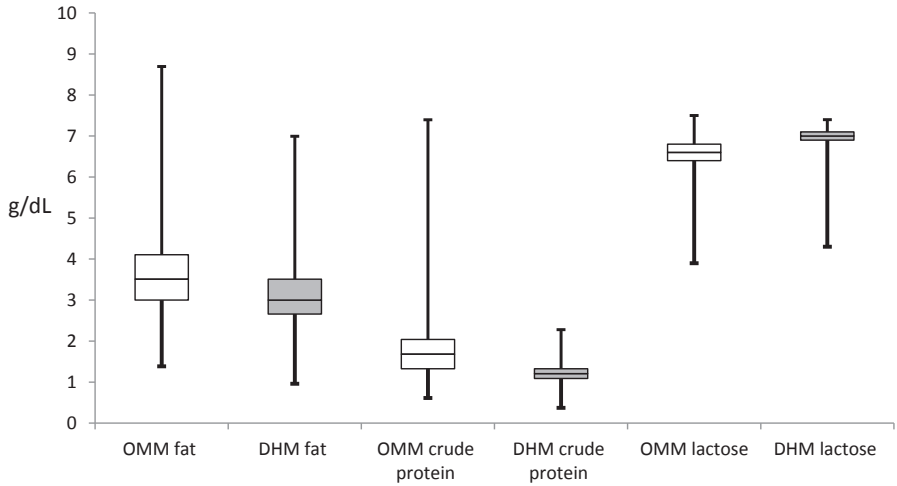

Fig. 1. Crude protein, fat, lactose, and energy content (median, P25, P75, minimum, and maximum values) assessed by a near infrared analyzer (Miris 3rd generation, Sweden) in MOM (N = 1350 samples) and DHM (N = 860 samples) treated in Auvergne Rhone-Alpes Regional Human Milk Bank. Twelve particularly high protein values above 4g/dL, obtained in OMM from twelve different mothers, 24 to 40 days after delivery. OMM, own mother's milk.

gain during hospitalization.[16] Thus, DHM is able to support postnatal growth, as fresh milk, as soon as it is fortified appropriately.

There are data suggesting an association between the consumption of HM during hospitalization and further cognitive development of very low-birth-weight infants.[2,32] Furthermore, it has been shown that, in premature infants exhibiting poor postnatal growth (weight-for-age *z*-score at −1.4 SD at discharge), cognitive development is better if they received breast milk during hospitalization.[33] Heat treatment applied to donor milk does not affect its fatty acid profile.[34,35] To date, there are no studies comparing the psychomotor development of preterm infants fed fresh versus pasteurized milk, but there is no rationale suggesting that it could impact long-term development.

Therefore, from the evidence available, it can be considered that DHM is beneficial for health and development of preterm infants. It is absolutely essential to provide preterm recipients with a safe and high-quality DHM, that is, without pathogens and preserving as much as possible its immunologic and nutritional properties.

MILK BANKS TO DELIVER SAFE AND HIGH-QUALITY DONOR HUMAN MILK

The first HMB opened in Vienna (Austria) in 1909 and soon after in Boston, Massachusetts, in 1910. In Europe, most HMBs opened during the second part of the twentieth century, and there are now 210 banks in 2016 (http://www.europeanmilkbanking.com). After the closing of many HMBs in North America during the 1980s, due to AIDS, they are now reopening, and the number is regularly increasing in North America and worldwide (https://www.hmbana.org/locations).

HMBs are established to recruit and screen breast milk donors, collect, treat (bacteriologic screening, pasteurization, storage), and distribute the donated HM. In most countries, it concerns only DHM, but in some countries, it concerns also MOM, notably when the mother is positive for cytomegalovirus (CMV), when the collection

has not been performed in good hygienic conditions, or when the milk has been stored for more than 48 to 96 hours[36–38] (Fig. 2). Another role of HMBs is to promote/support breastfeeding in mothers of hospitalized preterm infants and among prospective donors.

HMBs rely on a donor breastfeeding population to ensure adequate supply. Donors are mothers who delivered often at term and give their extra milk for vulnerable hospitalized babies. Sometimes mothers who delivered preterm and produce enough milk to cover the needs of their own baby want to give the milk surplus to the HMB when their baby is discharged to home (Fig. 3). The DHM will be given to hospitalized preterm infants under medical prescription. This altruistic act, based on the

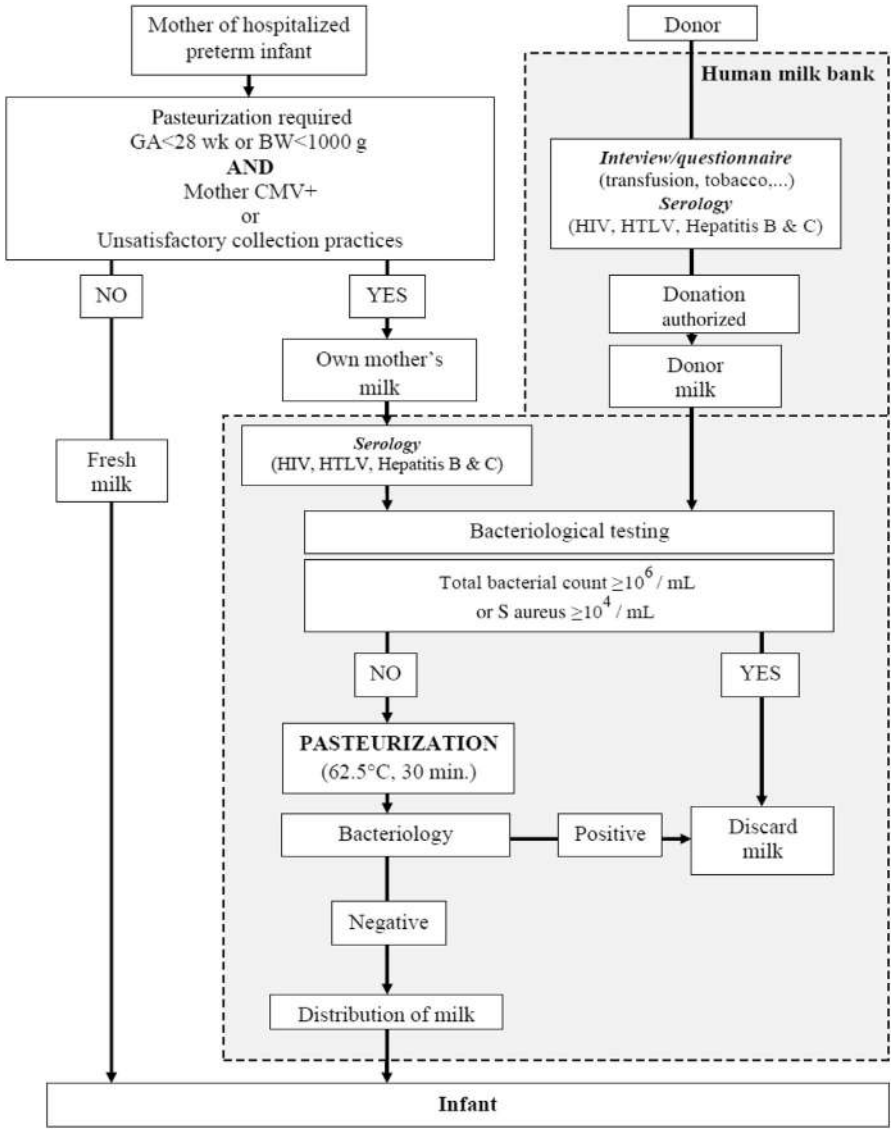

Fig. 2. HMBs and feeding preterm infant with HM. BW, birth weight; GA, gestational age.

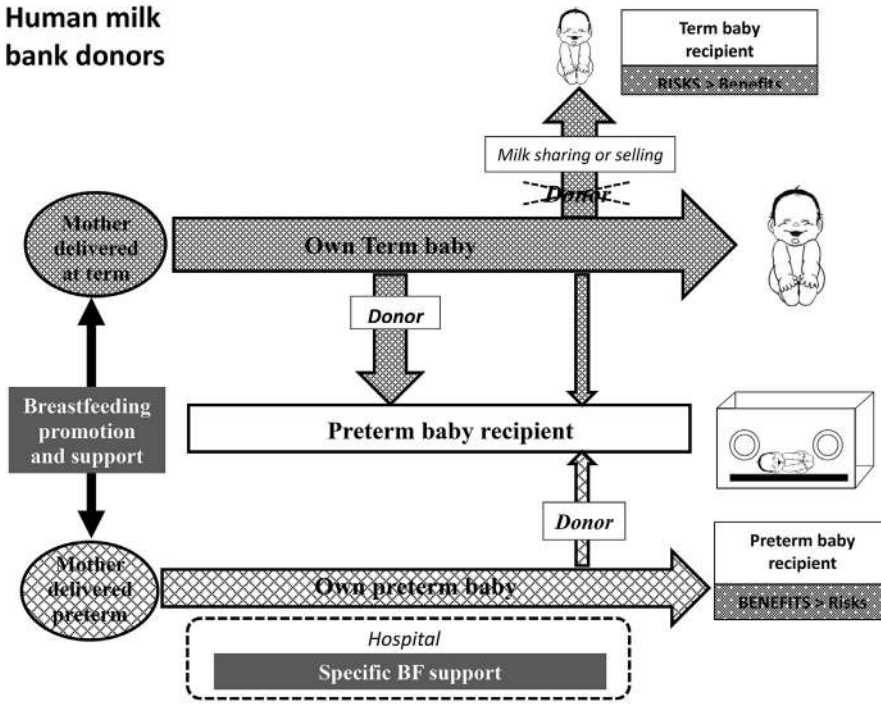

Fig. 3. Preterm baby can receive MOM or DHM. BF, breast feeding.

comprehension of the specific benefits of HM for preterm infants, is completely different from the mothers who give (or sell) their extra milk to another mother who delivered a healthy term baby who cannot get enough milk from his/her mother (see Fig. 3). The latter should not be considered "donors," which is applicable only for mothers giving their extra milk to HMBs to help sick babies and therefore have been carefully screened for health problems. Furthermore, some critical issues with bacteriologic safety and cow's milk protein content in such exchanged milk have been recently reported.[39–42]

To provide safe, high-quality DHM, a high-level quality assurance and strict tracking are required in HMBs. As the number of HMBs expands worldwide, universal common core practices are required, but there is not yet enough evidence for each practice, which leaves the field open to a significant heterogeneity.[43] More and more work is being done in each country to elaborate consensus statements based on evidence, otherwise on experience.[38,44–51] It concerns "traditional" milk banks, but other types of milk banks are settled over the world. Some "milk banks" are organized to collect, store, and distribute MOM.[52,53] These milk banks are installed in-hospital and are working only to provide infants hospitalized in the same hospital with MOM. In some countries such as Muslim countries, it may be difficult to build HMBs because of religious and cultural fear about milk kinship, and a model of HMB appropriate for these settings has been proposed.[54] In Brazil, there are more than 200 mother/baby centers for donor milk donation and lactation support. In settings where there is no access to pasteurizers, flash pasteurization has been proposed.[38] However, only the treatment and quality of milk treated in "traditional" milk banks are discussed later because it is the most common model.

To deliver safe and high-quality DHM, care must be taken in the process at all steps: selection of donors, collection, storage, treatment, and distribution. For example, the donors should be well informed to understand their responsibility in avoiding bacteriologic contamination, and therefore, milk discarding in the HMB due to bacteriologic count above the limit.[45,47,51] The present article discusses the treatment of HM. For the other steps, see Ben T. Hartmann's article, "Ensuring Safety in Donor Human Milk Banking in Neonatal Intensive Care," in this issue.

Treatment of HM should inactivate bacteria, viruses, and other potential pathogens while limiting the impact on the milk's protective elements or nutrients, such as proteins, antibodies, enzymes, and vitamins. Furthermore, the method should be usable in the setting of HMBs treating significant amounts of milk each day. To date, the best compromise is represented by Holder pasteurization, but new techniques have been proposed recently and will probably become available in the next years.

TREATMENT OF HUMAN MILK IN MILK BANKS

Low-Temperature Long-Time Pasteurization

The most common practice is a low-temperature (62.5°C) long-time (30 minutes), pasteurization (LTLT) known as "Holder" pasteurization. Pasteurization has long been the standard method to extend the shelf-life of dairy products and to reduce the risk of food-borne pathogens. This technique was first published by Nicolas Appert in 1831, developed by Louis Pasteur in 1865 for the pasteurization of beer, and used by the dairy industry to protect the bovine milk from *Mycobacterium bovis*. The milk was heated in trays that were called "Holders."

As LTLT offers the best compromise between microbiological safety and preservation of some important milk components, most of HMBs worldwide uses this reference technique.[38,44–51]

Impact of LTLT on components useful for nutrition and anti-infectious properties of HM has been extensively evaluated, mostly in experimental conditions.

Effect of low-temperature long-time pasteurization on microorganisms, bactericidal activity, cytokines, immunoglobulins, lactoferrin, and lysozyme

Safe HM from the HMB should not contain pathogens but should also retain antibacterial capacity and preserve immune proteins such as immunoglobulins, lactoferrin, and lysozyme contributing to the protection of the neonate against infection. HM has its own microbiota, which supports the development of gut microbiota in term infants.[55] However, in preterm infants with an immature immune system, it is not acceptable to propose HM-containing pathogens.[56] LTLT kills most pathogenic bacteria found in breast milk.[57] LTLT does not destroy sporulated bacteria such as *Bacillus cereus*, which is pathogenic in immunocompromised patients, including preterm infants.[58,59] It has been shown that *B cereus* spores in raw milk are the major source of *B cereus* in pasteurized milk.[60] Detection of *B cereus* in pasteurized milk justifies postpasteurization testing for any flora and, to the authors' knowledge, no severe infection related to pasteurized milk has been reported, even if suspected.[61] The main source of *B cereus* is usually found in the environment of infected or contaminated preterm infants.[62,63]

As heavily contaminated milk can theoretically contain enterotoxins and thermostable enzymes even after pasteurization, most HMBs test for total viable microbial content and other undesirable microbes such as *Staphylococcus aureus* (± Enterobacteriaceae in some countries) before pasteurization.[64] Milk is discarded when total viable microorganisms are greater than 10^4 colony forming units (CFU)/mL, *S aureus* greater than 10^4 CFU/mL, and Enterobacteriaceae greater than 10^4 CFU/mL (**Table 1**). Most countries recommend also microbiological testing after pasteurization, to monitor for

Table 1
Testing for microbial contamination of human milk before pasteurization in human milk banks according to guidelines published in 5 different countries

France	UK	North America	Italy	South Africa
2008	**2010–2012**	**2013**	**2008–2010**	**2011**
Testing of *subbatches* (donations from the same donor) and *batches* • In some HM banks, *sub-batches are tested* for total aerobic flora and kept in quarantine (+4°C) while waiting for the results. ◦ Total aerobic flora $\geq 10^6$ CFU/mL? ■ Yes → Subbatches are destroyed ■ No → Subbatches are grouped in batches and tested for total aerobic flora and *S aureus* before being pasteurized: ◦ If total aerobic flora $\geq 10^6$ CFU/mL or *S aureus* $\geq 10^4$ CFU/mL? ■ Yes → discard batches ■ No → deliver batches • In some HM banks, *subbatches are not tested*, and then batches undergo the same bacteriologic testing before being packed in bottles and pasteurized. The batches are discarded if total aerobic flora $\geq 10^5$ CFU/mL or *S aureus* $\geq 10^4$ CFU/mL. In all cases, while waiting for the results of testing, the milk is kept at + 4°C for 48 h or pasteurized immediately, frozen, and kept in quarantine until its compliance has been proven.	Test a sample from each batch of pooled donor milk for microbial contamination and discard if samples exceed a count of: • Total viable microorganisms $\geq 10^5$ CFU/mL or • Enterobacteriaceae $\geq 10^4$ CFU/mL or • *S aureus* $\geq 10^4$ CFU/mL	No testing	Testing • At the first donation • When the donor does not seem to guarantee the appropriate hygienic conditions • In any case, periodically in a random manner The milk is discarded if it contains: • Total viable bacteria $>10^5$ CFU/mL or • Enterobacteriaceae $>10^4$ CFU/mL or • *S aureus* $>10^4$ CFU/mL	Testing for microbial contamination should be done on an aliquot of milk from the first donation of each donor mother. If any contamination is found, milk must be discarded and milk from the donor mother must be rechecked with the subsequent donation. For community-based banks, a simpler test which is used by all the Brazilian Breastmilk Banks is known as titratable acidity

contamination introduced after the pasteurization process or pasteurization failure. Safety of donor milk recipients is ensured by discarding milk if any microbial content is found (**Table 2**). There is no consensus on bacteriologic methods, but it is recommended to discard milk with any pathogen as the anti-infective properties of HM are modified by heat treatment. Such prepasteurization and postpasteurization microbiological screening results in approximately 10% to 30% of milk being discarded.[65,66] A strict application of the Hazard Analysis Critical Control Point principles during collection, storage, and pasteurization of donor milk is useful to reduce the amount of milk discarded.

Such microbiological testing of HM ensures bacteriologic safety of the final product delivered to preterm babies. Viral safety is ensured by selecting the donors through an interview and by testing the mothers for pathogenic viruses (HIV, hepatitis B and C, human T-cell leukemia virus type [HTLV]) before accepting that they can donate their milk. This screening is particularly important because LTLT destroys most high-risk viruses such as HIV, CMV, human papillomaviruses, herpes viruses, HTLV, but not hepatitis B virus.[67–72]

LTLT leads to the loss of some biologically active milk components and cells that are crucial for the defense against infections, such as immunoglobulins, leukocytes, secretory immunoglobulin A (IgAs), total IgAs, IgM, IgG, lactoferrin, lysozyme, lymphocytes, and cytokines.[20,73–75]

After LTLT around 40% of IgA is retained.[74–78] However, other studies have presented a higher IgA retention, ranging between 60% and 80%,[68,79–83] and even 100% was reported by some investigators.[83] Summary of main effects of LTLT on milk components is presented in **Table 3**.[84–113] These discrepancies could be explained by several factors, such as the time for reaching exact temperature, the time for cooling, and the volume of HM processed. Indeed, in most experimental studies that evaluated the effect of LTLT, only small aliquots (40 μL to 10 mL) were treated,[21,75,76,113] which is still much lower than the volume of milk usually processed in HMBs (100–250 mL). In such real conditions, higher loss of HM components is expected to occur due to the longer time needed to reach the desired temperature in the center of the container. It is crucial to ensure that the exposition of milk to high temperature is not too prolonged, because it could alter immunologic properties of milk.[80] Different devices are marketed to perform LTLT pasteurization, using water in different conditions, or air. The exposure to high temperature may be prolonged and/or excessive with some devices (Buffin 2016, personal data). Therefore, each pasteurizer should undergo regular quality control performed by each HMB to be sure that the increase in temperature or the temperature plateau is not too prolonged and that cooling is rapid enough. It requires the presence of temperature probes in some of the milk bottles undergoing pasteurization. The number of probes and where they have to be positioned into the pasteurizers are not clearly defined by the manufacturers, and until recently, some manufacturers did not even propose any probe in their device. Based on published data and daily experience of the 36 milk banks over the French territory, the French Human Milk Bank Association recently proposed that much stricter quality control should be done regularly on pasteurizers (**Fig. 4**). It should be noted that most studies evaluating the effect of pasteurization on microbial or immune content in HM used in vitro methodology based on special devices to mimic the pasteurizer, but it is required to perform this type of study in real conditions, using pasteurizers that underwent quality control. When studies were performed using pasteurizers, no study presented any information about the quality control of the device used.

Lactoferrin is bacteriostatic because it inhibits the growth of iron-dependent pathogens by reducing free iron availability, but it is also bactericidal through bactericidal

Table 2
Testing of pasteurized human milk for microbial contamination in human milk banks according to guidelines published in 5 countries

France	United Kingdom	USA & Canada	Italy	South Africa
2008	**2010–2012**	**2013**	**2008–2010**	**2011**
Systematic testing for any microbial contamination Test: 0.5 mL of undiluted milk on blood agar and incubation 48 h at 37°C. • Any batch with positive testing is destroyed. • A documented analysis is performed in order to find the causes of recurrent contamination. • Milk bottles are placed in quarantine after pasteurization and cooling and while waiting for the results of the testing. • Milk bottles can be either stored at +4°C for no more than 48 hours and frozen at −18°C or frozen immediately at −18°C.	*Regular testing* • At least once a month or every 10 cycles, depending on which comes first, or • On an ad-hoc basis if any new processes, equipment, or staff are introduced, or if there are concerns about any part of the process.	Bottle of milk used for testing should be chosen *randomly* and discarded after use Entire bottle of milk randomly selected from a batch or sample aliquots (100 μL of milk on sheep blood agar plates or plate count agar, results in CFU/100 μL) Any bacteriologic growth is unacceptable for heat-processed milk Aerobic or standard plate <1 CFU/100 μL • Dispense >5 CFU/100 μL • Discard Between 0.5 and 5 CFU/100 μL • "Indeterminate" Test 2 additional bottles from the batch (same procedure) If negative: Dispense If positive: Discard	*Regular testing* • Once a month, or • Every 10 pasteurization cycles, or • When there are concerns about the processing. The milk must be discarded in case of any kind of bacterial growth.	*Systematic testing* for microbial contamination. Test: 1 mL of milk: enumeration of E coli and coliform colonies on Petrifilm EC plates. Test: 100 μL of each sample to be plated onto McConkey agar and incubated for 24 h at 37°C. The following day a semiquantitative count will be performed. Only if required, will any suspicious colonies be further identified. If any contamination is found, milk must be discarded and milk from the donor mother must be rechecked with the subsequent donation.

Table 3
Summary of main effects of low time low temperature ("Holder") pasteurization on milk components

Component	→	↑	↓	∅	Ref.
Proteins					
Total protein content	X	—	—	—	84,85
Protein quality					
Available lysine	—	—	X[a]	—	86,87
Free amino acids					
Cystine, taurine, methionine,	X	—	—	—	88,89
Arginine, leucine, glutamine	—	X	—	—	88,89
Aspartate	—	—	X	—	89
Bioactive peptides	X	—	—	—	26
Enzymes					
Amylase	—	—	X	—	68,87,90
Lipase, LPL, alkaline phosphatase	—	—	—	X	90
Immunoglobulins					
IgA, IgAs, IgG, IgM IgG4	—	—	X	—	77,82,91–95
Lactoferrine	—	—	X[a]	—	79,80,83,92,93,95
Lyzozyme and lyzozyme activity	—	—	X[a]	—	68,74,76,77,80,82,83,92,95,96
Lipids					
Total fat	X	—	—	—	30,34,84,85,90,97
Free fatty acids	—	X	—	—	30
Fatty acids 18:1, 18:3, 12:0, 14:0, 18:0	X[a]	—	—	—	34,35,89,90,98–101
Other fatty acids	X	—	—	—	30,98,100,101
Saccharides					
Glucose	X[a]	—	—	—	94,102
Lactose, oligosaccharides, GAG	X	—	—	—	84,85,94,97,102–104
Vitamins					
E, B2, B3, B5, B12, Biotin	X	—	—	—	93,105
C, D, B6	—	—	X	—	99,106,107
A, C, α-γ-δ-tocopherols	—	—	X[a]	—	35,94,97,100,108
Zinc, Copper, Iron	X	—	—	—	97,109
Growth factors					
EGF, TGF-ß1, TGF-ß2, MCP-1	X	—	—	—	20,94,110
GM-CSF	—	X	—	—	94
EPO, HB-EGF, IGF-1, IGF-BP∼2 & ∼3	—	—	X[a]	—	97,100,108
Hormones					
Leptine	X	—	—	—	79
Insuline, adiponectine	—	—	X	—	111
Cytokines					
IL-2, -4, -5, -12, -13, -17	X	—	—	—	35,94,100
IL-8, -7	—	X[a]	—	—	94,100
IL-1ß, -6, -10, TNF-α, INF-γ	—	—	X[a]	—	35,94,108

(*continued on next page*)

Table 3
(*continued*)

Component	→	↑	↓	∅	Ref.
Oxidative stress markers					
Malonedialdehyde	X	—	—	—	112
Glutathione, GPA, TAC	—	—	X	—	112
Bactericial activity	—	X	—	—	113
Cells					
Lymphocytes	—	—	X	—	91
Macrophages	—	X	—	—	96
Electrolytes and minerals	X	—	—	—	78
Osmolality	X	—	—	—	78

Abbreviations: →, No/minor change; ↑, increase; ↓, decrease; ∅, Destruction; GAG, glycaminoglycans; GPA, glutathione peroxidase activity; LPL, lipoprotein lipase; TAC, total antioxidant capacity; TNF, tumor necrosis factor.

[a] Discordant results; requires further studies.

peptides resulting from the digestion of lactoferrin. There are some discrepancies in the results of studies that evaluated the effect of LTLT on lactoferrin. Depending on the study and the technique used for measurement of lactoferrin concentration, it was shown that about 10% to 65% of lactoferrin is retained after

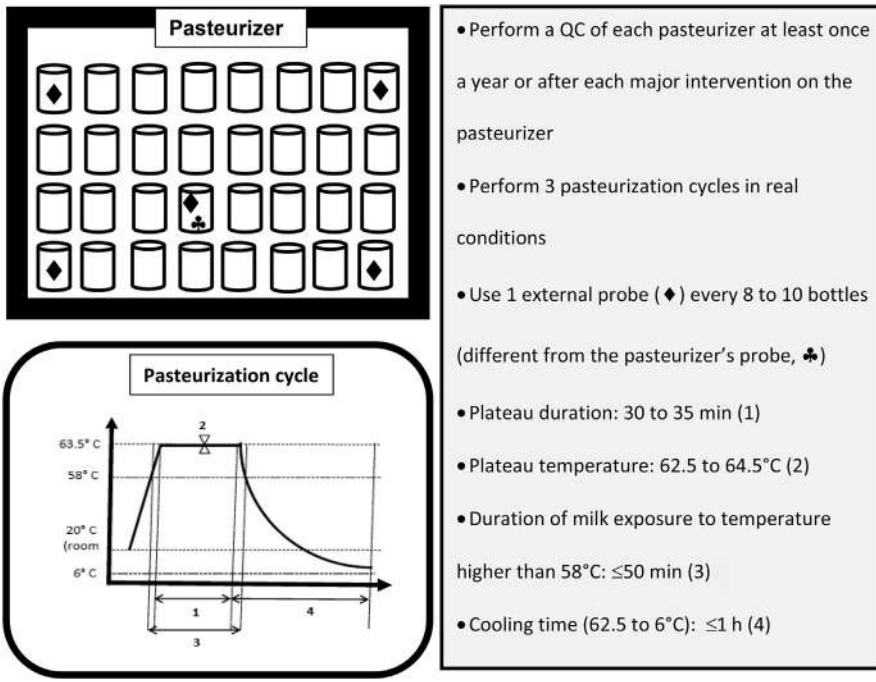

Fig. 4. Quality control (QC) parameters to be performed on each pasteurizer device used in HMB according to the French Human Milk Bank Association.

LTLT.[79,80,83,92,93,95] Therefore, there are still unanswered questions about lactoferrin, but it seems that some lactoferrin remains after LTLT, and part of the activity could be retained in pasteurized HM despite reduction of its concentration (see **Table 1**).

Lysozyme has a very stable structure maintained by disulfide bonds and is therefore highly resistant to technological treatments. Most studies report that lysozyme activity is maintained after LTLT, but the proportion of lysozyme retained is quite variable (15%–80%).[68,76,77,79,80,82,83,92,114] Similar discrepancies were reported in regard to lysozyme activity[77,92] (see **Table 1**).

HM possesses bactericidal capacity that is reduced after thermal processing. Bactericidal capacity (against *Escherichia coli*) is preserved after LTLT pasteurization, but is less than in untreated milk. *E coli* growth is reduced 52% versus 70% in untreated milk.[113] Bactericidal capacity is, moreover, better preserved by LTLT than when using higher heating temperatures[113] (see **Table 1**).

Most cytokines in HM are anti-inflammatory and theoretically could help preterm infants presenting with infections. After LTLT, most cytokines are unaffected; some increase, and few decrease[35,94,100–108] (see **Table 1**). Clinical relevance of such a cytokine profile is unknown.

Effect on nutrients (protein, lipids, carbohydrates, vitamins), enzymes (lipase), antioxidant capacity, growth factors, and antioxidant capacity

Protein and energy (total fat, and lactose) are preserved or slightly reduced after LTLT. Oligosaccharides, vitamins A and E, lactose, long chain polyunsaturated fatty acids and epidermal growth factor are preserved by LTLT.[20,73,90,103,105,112] Recently, Gomes and colleagues[106] reported a small (10%–20%) reduction of the concentration of some vitamin D compounds after LTLT. After LTLT, there is a significant decrease in the concentrations of ascorbic acid plus dehydroascorbic acid, ascorbic acid, α-tocopherol, and γ-tocopherol.[99,107] Iron, copper, and zinc are slightly reduced by LTLT, but the concentration remains within the acceptable range required to cover the specific nutritional needs of newborn infants fed pasteurized HM[109] (see **Table 1**).

LTLT leads to the loss of lipase, alkaline phosphatase, and some growth factors.[20,73,112]

Milk fatty acids, including polyunsaturated long-chain fatty acids, are unaffected by LTLT, but completely inactivate the enzyme lipase.[90] BSSL is required for fat absorption by the small intestine. As the infant can synthetize and secrete small quantities of BSSL, the BSSL present in HM acts to supplement this deficiency. BSSL is inactivated at relatively low temperatures (45–55°C).[98] BSSL activity is completely lost after Holder pasteurization[87,115] (see **Table 1**).

LTLT has no effect on total protein content of HM, but could have an effect on protein quality, as reflected by a significant (−29%) decrease in available lysine content after HTST when compared with fresh milk.[86] In an additional study using a different technique to apply HTST, this finding was not confirmed.[87] Thus, further studies are requested. However, it has been shown that LTLT pasteurization does not affect protein digestibility because the profile of bioactive peptides released from HM proteins by gastrointestinal digestion was preserved after pasteurization[26] (see **Table 1**).

Antioxidants are present in HM[116,117] and are part of the natural defense system against the action of free radicals, which are related to necrotizing enterocolitis, retinopathy of prematurity, bronchopulmonary dysplasia, and other complications of prematurity. The total antioxidant capacity of HM is decreased after LTLT pasteurization due to the decrease in some components like glutathione[112] of HM implies a decrease in total antioxidant capacity of HM (see **Table 1**).

Other Techniques to Pasteurize Human Milk

New techniques consist of a thermal (high-temperature short-time pasteurization [HTST] and thermoultrasonic pasteurization), or nonthermal (high-pressure processing [HPP] and UV irradiation) pasteurization.

Higher-temperature short-time pasteurization

HTST may be more effective at retaining milk properties. The most frequently evaluated is HTST using a temperature of 72°C for 15 seconds,[68,86,87,93,113] but different temperatures (62–82°C) for different times (1–19 seconds) have been tested on HM.[20,118–120] Different experimental systems were used to obtain HTST[68,93,118,119] but have never been tested in HMB conditions, as there is not yet a marketed device.

Effect of higher-temperature short-time pasteurization on microorganisms, bactericidal activity, cytokines, immunoglobulins, lactoferrin, and lysozyme HTST is efficient to eradicate bacteria (*E coli*, *S aureus*, *Staphylococcus agalactiae*, coliforms, and total viable count), CMV, and enveloped viruses (HIV, hepatitis B and C).[68,118–120] Nonenveloped viruses, such as hepatitis A are not eradicated by HTST.[120]

In experimental conditions, the concentration of lactoferrin is significantly reduced following HTST,[76,87,93] but the proportion of lactoferrin retained is still twice higher (52% after 600 MPa for 15 minutes) than after LTLT pasteurization (20%).[76]

After HTST, around 40% IgA is retained, as reported after LTLT pasteurization.[76] Similar retention for IgG, but total destruction of IgM, was reported.[93,119]

The residual enzymatic activity of lysozyme is higher or maintained when compared with untreated HM.[76,118] One study using different HTST technique reported a decrease in lysozyme.[68]

HTST was reported to have no effect on HM cytokines.[20]

Bactericidal capacity of HM (against *E coli*) is preserved after HTST, but is less than in untreated milk and in milk treated by LTLT. *E coli* growth is reduced by 36% after HTST versus 70% in untreated milk.[113]

Effect on nutrients (protein, lipids, carbohydrates, vitamins), enzymes (lipase), antioxidant capacity, growth factors, and antioxidant capacity Similar to LTLT pasteurization, the HTST technique was first shown to inactivate the BSSL,[118] but a recent study suggests that the integrity of the BSSL and its activity are preserved after HTST.[87]

HTST was reported to have no effect on total protein, folic acid, vitamin B12, and vitamin C.[63,86,118] Some investigators reported an effect on protein quality as reflected by a significant, but modest (−14%), decrease in available lysine content after HTST when compared with fresh milk.[86]

HTST, like LTLT, reduces significantly the amount of alkaline phosphatase and lipase in HM.[63]

Total antioxidant capacity of HM is preserved after HTST.[112]

In summary, experimental data suggest that HTST is efficient to destroy most microorganisms, as LTLT, but is better at preserving biological quality of HM. It should be confirmed in HMB conditions.

High-pressure processing

HPP has been used to inactivate pathogenic microorganisms in solid and liquid foods.[121–123] This technology applies high hydrostatic high pressure (usually 400–800 MPa) for a short time. Changes in the chemical composition, color, and aroma related to pressurization are less than those caused by a heat treatment.[122] Different levels of pressure (200–900 MPa), during different times (1–120 minutes), were tested on HM.[35,75,77,81,82,107,119,124,125]

Effect of high-pressure processing on microorganisms, bactericidal activity, cytokines, immunoglobulins, lactoferrin, and lysozyme The effect of HPP depends on the type of bacteria. Gram-positive microorganisms are more resistant to pressure than gram-negative ones, but pressure resistance varies also within strains of the same species. The pressure required to achieve inactivation of gram-positive microorganisms (500–600 MPa) is higher than for gram-negative microorganisms (300–400 MPa). Vegetative forms of yeasts and molds are the most pressure sensitive.[126] Many studies on the inactivation of microorganisms by HPP have been performed in milk during the last few years and have generally demonstrated that it is possible to obtain milk with a microbiological quality comparable to that of milk pasteurized at 72°C for 15 seconds, depending on the microbiological quality of the milk,[127] but not comparable to sterilized milk due to HPP-resistant spores. The inactivation of bacterial spores using HPP conditions is very difficult.[128]

Not all the results obtained using this new technology are presently available because of pending patenting process, but preliminary data suggest that HPP, performed in specific conditions, could destroy vegetative flora, *S aureus*, bacterial spores, and viruses together with a much better preservation of lactoferrin, lysozyme, and lipase than after LTLT.[129]

Because there is a high level of structural diversity among viruses, the effect of pressure is variable.[126] Complete inactivation of hepatitis A virus can be achieved by treatment at 275 to 450 MPa for 5 minutes.[130] As pressure at 300 MPa damages the virus envelope and prevents the virus particles from binding to the cells, it has been suggested that HPP could inactivate Herpes simplex virus-1, CMV, and other enveloped viruses.[131–133] HPP inactivates HIV.[134]

There are very few studies published on the efficacy of HPP processing on HM, and the first report about HPP ability to inactivate 5 selected bacterial pathogens in HM is quite recent.[135] The efficacy on bacteria seems to be variable depending on pressure applied and duration of exposure to high pressures. The efficacy of HPP is great on *Listeria monocytogenes*, Enterobacteriaceae, and total viable count that are eradicated, but is less for *E coli* and *S aureus*.[81,135]

The effect of processing on the immune cells (leukocyte content) and immunoglobulins content (IgM, IgA, and IgG) was evaluated.[75] HPP (400 MPa for 3 or 6 minutes) maintained the original levels of immunoglobulins and preserves leukocytes of HM better than LTLT.[75] Only the treatment less than 300 MPa maintains certain levels of immunoglobulins (75% of IgM, 48% of IgA, 100% of IgG).[125]

In experimental conditions, after the application of pressures between 300 and 650 MPa for 30 minutes, 36% to 80% of IgA is retained, whereas it is 40% after LTLT.[76,77] In similar conditions, lysozyme activity increased by 20% to 40%, which is approximately the same as after LTLT pasteurization.[76,81] After the application of pressures between 300, 400, 500, and 600 MPa for 15 minutes, 91%, 77%, 66%, and 52% of lactoferrin was retained, whereas it was 20% after LTLT.[136]

HHP induces a minimal effect on the level of interleukin-12 (IL-12), IL-17, and interferon-γ (IFN-γ), but leukocytes are very sensitive to HHP.[125]

Effect on nutrients (protein, lipids, carbohydrates, vitamins), enzymes (lipase), antioxidant capacity, growth factors, and antioxidant capacity HPP influences the physicochemical properties of milk. It has significant effect on protein quality. HPP denatures whey proteins, affects the activity of native milk enzymes, and produces changes in casein micelles, with the subsequent solubilization of colloidal calcium phosphate.[137,138] In milk subjected to HPP, the casein micelles are disintegrated into smaller particles, which are accompanied by an increase of caseins and calcium

phosphate levels in the serum phase of milk.[139] After pressure treatment up to 500 MPa at 25°C, β-lactoglobulin is the most easily denatured serum protein, and denaturation of the immunoglobulins occurs at the highest pressures and particularly at 50°C. Greater than 300 MPa, there is a significant impact on the structure of β-lactoglobulin.[140]

There are discrepancies about the effect on fatty acids. Moltó-Puigmartí and colleagues[107] reported no effect of high pressures (400–600 MPa), but another study reported that the content of fatty acids is affected by the highest pressure values (600 and 900 MPa).[125]

No changes in lactose were observed after pressurization (100–400 MPa for 10–60 minutes at 25°C), suggesting that no Maillard reaction or lactose isomerization occurs in milk after pressure treatment.[141]

Vitamin C and tocopherol are retained after HPP, contrary to LTLT pasteurization.[107]

In summary, experimental data suggest that HPP is efficient to destroy most microorganisms, as LTLT, but could be better at preserving biological quality of HM. It should be confirmed in HMB conditions.

Ultraviolet irradiation

UV irradiation, specifically UV-C, destroys microorganisms, such as bacteria, viruses, and yeasts, but its penetration capacity is low, which limits its use to liquid foods and flat surfaces.[142,143] Data are mostly experimental, but it has never been used in HMB to pasteurize large amounts of milk. Ultrasound processing or sonication is one of the alternative technologies that have been proposed for food processing with a reduced impact on nutritional content and overall food quality. Sonication alone is not very effective in killing bacteria in food and has been coupled with mild heating (thermoultrasonication).[144] A 2- to 8-minute treatment at 50°C of artificially contaminated HM inactivates bacteria such as *E coli* and *Staphylococcus epidermidis*, with a retention of secretory IgA, lysozyme, lactoferrin, and BSS: of 91%, 80%, 77%, and 45%, respectively,[144] which is lower than the approximately 20% retention for observed secretory IgA, lysozyme, lactoferrin, and 0% for BSSL, after Holder pasteurization. It also inactivates CMV.[145]

For the ultrasound processing and UV irradiation, data are mostly experimental, and the feasibility of routine use in an HMB has yet to be demonstrated. Although LTLT pasteurization is the reference method, both HPP and HTST are promising as devices that could be usable in the near future in the setting of HMB.[82,129,146]

Pasteurization method used depends on organization and financial resources. Some simple, low-cost techniques such as flash-heat pasteurization have been proposed in resource-limited settings.[147–150] The Human Milk Banking Association of South Africa recommends that, where there is no access to pasteurizers, milk can be pasteurized using the following method: Up to 120 mL of milk should be expressed into a clean 450- to 500-mL glass jar, placed in a 1-L aluminum Hart pot and cold water added sufficient to cover the level of milk by 2 finger widths. The pot containing water and jar should be placed in the middle of the heat source. After water reaches a rolling boil, the jar with the breast milk should be removed and allowed to cool. Flash heat should preferably be performed as soon after expression as possible, and then HM can be stored for 8 hours at room temperature.[38]

OTHERS POINTS RELATED TO THE QUALITY OF DONOR HUMAN MILK

Absence of Cow's Milk Protein in Banked Milk

Some HMBs test for the presence of cow's milk protein, because it happens that mothers add cow's milk to their own milk. However, there is a lack of a simple, low-

cost, and efficient test. Furthermore, it seems to be particularly relevant outside the setting of HMBs, when mothers get money by selling their milk, but the issue of HM purchased via Internet is completely different from milk donation.[151] In most countries, donors are not paid or receive small financial compensation,[47,50] so that there is no real temptation to add cow's milk to their milk. As the test was used in France between 1999 and 2009, French HMBs Association performed a survey and was able to estimate that about 99,500 tests were performed in 17 HMBs during this period of time and less than 10 tests were positive (personal data). These cases were related to maternal psychiatric troubles, and the amount of cow's milk was so important that professionals working in the HMB easily detected the unusual white aspect of milk when compared with HM. Furthermore, preterm infants receive significant amounts of cow's milk protein because cow's milk–based fortifiers are widely used and well tolerated in most preterm infants. Some studies have suggested that HM-based fortifiers could help to improve digestive tolerance and reduce the risk of necrotizing enterocolitis, but these products are not widely available and are still very expensive. Furthermore, their efficacy is still to be demonstrated in settings where the basal risk of necrotizing enterocolitis is low (3%–5%).[151,152] However, the detection of cow's milk protein in DHM does not seem to be relevant anymore in HMBs.

Labeling Nutritional Content of Banked Milk

Assessing macronutrient content of DHM can be useful because HM composition is variable (see Francis B. Mimouni and colleagues' article, "Preterm Human Milk Macronutrient and Energy Composition: A Systematic Review and Meta-Analysis," in this issue), and not to assess the effect of pasteurization on nutrients' content of HM because it is very limited (see earlier discussion). The effect of pasteurization is much lesser than the effect of the mode of feeding (continuous, bolus), which may have a huge effect on fat content of HM. Fat losses have been reported to be as high as 50%.[84] Therefore, it is crucial that the staff supports suckling and breastfeeding in preterm infants. The earlier these babies will suck efficiently from breast, the earlier the feeding tube and syringes will be removed, which is crucial to avoid fat losses. Bedside assessment of milk composition has been proposed because of the great variability of HM protein and energy contents (see **Fig. 1**), because bedside techniques became available (see Gerhard Fusch and colleagues' article, ""Bed Side" Human Milk Analysis in the Neonatal Intensive Care Unit: A Systematic Review," in this issue), and because products such as specifically designed protein supplement became available to perform a targeted fortification of HM (see Francis B. Mimouni and colleagues' article, "The Use of Multinutrient Human Milk Fortifiers in Preterm Infants: A Systematic Review of Unanswered Questions," in this issue). In some facilities, milk is tested for nutritional content every 2 weeks, each day, or even more to perform individualized targeted fortification.[50,153] In most places it is not done yet due to a lack of resources (staff and reliable analyzers) and because there is no strong evidence, even if there are some indications from pilot studies that it could help.[153] However, as the DHM is a mature milk often collected after a few weeks of lactation, its variability is much less that the variability in protein and lipid content in milk from mothers who delivered preterm (see **Fig. 1**). There is a consensus to use individualized fortification, but no consensus on the way to do it: targeted or adjustable. The latest does not require prior milk analysis because it is based on the evaluation of individual protein status through measurement of serum urea.[154,155] However, assessment of milk composition in HMBs could be useful to propose a DHM labeled with information about its protein and energy contents.

SUMMARY

HMBs are essential for providing safe donor milk to vulnerable infants, such as very low-birth-weight infants. The collection, treatment, and distribution of donor milk require technical processes that are still differing depending on locations, organization, and resources. Universal core requirements and quality principles for all HMBs are required. These requirements are often based on consensus between health professionals in each country. Further research is needed to help build universally accepted recommendations.

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