Supplement Article

Recommendations for the establishment and operation of a donor human milk bank

Sertac Arslanoglu, Guido E. Moro, Paola Tonetto, Giuseppe De Nisi, Amalia Maria Ambruzzi, Augusto Biasini, Claudio Profeti, Luigi Gagliardi, Guglielmo Salvatori, and Enrico Bertino

In Europe, an increasing number of human milk banks (HMBs) collect donor human milk to feed preterm infants when their mother's milk is not available or not enough. Moreover, donor milk is a bridge to breastfeeding, with positive clinical and psychological advantages for both mother and infant. Italy, with 41 HMBs actively operating in 2022, has the highest number of HMBs in Europe. The process of human milk donation is complex, so activity of HMBs must be regulated according to well-established rules. The present recommendations have been prepared as a tool to standardize the organization, management, and procedures of HMBs operating in Italy and to determine the minimal essential requirements to establish new HMBs. This article covers all the aspects of human milk donation and human milk banking, including general recommendations, donor recruitment and screening, expression, handling and storage of donor human milk, milk screening, and milk treatment (pasteurization). A pragmatic approach was taken to drafting the recommendations. Items for which there was consensus or robust published evidence on which to base recommendations were included. When there were differences that could not be resolved by reference to published research, a statement of explanation based on the expert opinion of the authors (all members of the Italian Association of Human Milk Banks) was included. Implementation of these recommendations can contribute to promotion of breastfeeding.

INTRODUCTION

The particular composition of human milk (HM) makes it suited for the nutritional and biological requirements of the neonate. Breastfeeding, with only a few exceptions, is the norm in infant nutrition, conferring short- and long-term health benefits for the infants and the lactating mothers. Benefits of HM are well recognized not only for term, but also and particularly for preterm and sick infants.[1,2] HM feeding is

Affiliation: *S. Arslanoglu, G.E. Moro, P. Tonetto, G. De Nisi, A.M. Ambruzzi, A. Biasini, C. Profeti, G. Salvatori,* and *E. Bertino* are with the Italian Association of Human Milk Banks (Associazione Italiana Banche del Latte Umano Donato), Milan, Italy. *S. Arslanoglu* is with the Division of Neonatology, Department of Pediatrics, İstanbul Medeniyet University, School of Medicine, İstanbul, Turkey. *P. Tonetto* and *E. Bertino* are with the Neonatal Care Unit of the University, City of Health and Science Hospital, Turin, Italy. *L. Gagliardi* is with the Woman and Child Health Department, Azienda USL Toscana Nord-Ovest, Lucca, Italy. *G. Salvatori* is with the Donor Human Milk Bank, Pediatric Hospital Bambino Gesù, Rome, Italy.

Associazione Italiana Banche del Latte Umano Donato, Via Libero Temolo 4, 20126 MIlan, Italy.

Correspondence: *G.E. Moro*, Italian Association of Human Milk Banks, Milan, Italy. E-mail: guidoemoro@tiscali.it.

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essential for very-low-birth-weight (VLBW) infants, primarily during the first critical weeks of life. It has been shown that HM feeding in this group of tiny infants improves short- and long-term health outcomes and decreases mortality rates.[3] Preterm infants fed HM have protection against necrotizing enterocolitis (NEC), sepsis, retinopathy of prematurity (ROP), and bronchopulmonary dysplasia (BPD) while having better neurodevelopment and cardiovascular health outcomes in the long term.[3]

Mother's own milk (MOM) is the first choice for all neonates, yet many tiny preterm and critically ill infants might not receive sufficient breast milk in the early days of life. When MOM is not available or insufficient, donor human milk (DHM) is the best alternative, as recommended by American Academy of Pediatrics,[4,5] European Society for Paediatric Gastroenterology Hepatology and Nutrition,[6] and World Health Organization.[7,8] In this situation, these scientific associations recommend the use of DHM, particularly for VLBW infants, in combination with appropriate lactation support for the mother. Although some bioactive milk components are lost to varying degrees with the heat treatment methods widely used by milk banks, many other precious bioactive compounds are completely or partially preserved and are not found in preterm formulas.[3,9]

An HMB is a facility that selects, collects, screens, processes, stores, and distributes DHM prescribed for babies who are medically fragile (primarily VLBW infants). DHM is breast milk that has been expressed voluntarily by a mother and provided freely to an HMB. The activity of the bank and the donation should be nonprofit actions. The primary aim of an HMB is the promotion and support of lactation and breastfeeding. Diffusion of the culture of milk donation and appropriate use of the donated HM are the next aims.[3] Thus, continuum of the provision of HM for preterm infants via HMBs would contribute to decreasing the morbidity and mortality rates.

To regulate the activity of an HMB and to ensure the safety and quality of DHM given to the recipient, several national milk-banking guidelines[10–13] and European Milk Bank Association recommendations[14] are available. This article presents the updated version of the 2010 guidelines of the Associazione Italiana Banche del Latte Umano Donato (Italian Association of Human Milk Banks).[10]

Objectives and target users of the recommendations

The present recommendations (a revised version of the Italian recommendations published in 2010)[10] were prepared as a tool to optimize and standardize the functioning of existing human milk banks (HMBs) in Italy and to determine the minimal essential requirements to establish a new HMB. Organization and management of HMBs must not only ensure the safety and quality of the product[3] but also protect the milk bank staff and the donors.

Target users of these recommendations are health care workers of HMBs, personnel in neonatology departments and neonatal intensive care units (NICUs), and administrators of hospitals with an established HMB or with the intention to establish one.

Development process for the recommendations

The working group (hereafter, the Panel) responsible of these recommendations is a multidisciplinary team composed of neonatologists, a clinical dietitian, and an epidemiologist. All the components of this Panel have a professional experience with human milk banking. Additionally, specialists in the fields of immunology, hematology, transfusional medicine, and microbiology; operators of HMBs from Cesena, Florence, Lido di Camaiore, Milan, Rome, Turin, and Trento; healthcare professionals working in NICUs; and a representative of an Islamic community served as external consultants. Bibliographic research was performed using the databases MEDLINE, Embase, and CINAHL, and we also manually reviewed scientific journals, proceedings and other publications on these specific topics (human milk, donor human milk, human milk donation, human milk banks) written in English, French, and Italian. Furthermore, official statements of scientific societies and legislative documents were included.

For consensus development, an informal method based on discussions between the Panel members was used. The levels of evidence and the grades of recommendations were classified according to the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) definitions.[15]

Advantages of feeding with donor human milk

In the clinical context, it is not always feasible to test the biological superiority of DHM compared with formula in terms of randomized controlled trials (RCTs), which are the primary tools to evaluate the efficacy of an intervention. Most of the RCTs comparing 2 distinct groups fed DHM (fortified only in recent studies) vs formula (term or preterm) have some weak points: some include MOM, some do not specify whether the infants receive MOM or DHM, or their proportions and/or duration are not reported.[6,16]

Clinical outcomes evaluated until now include incidence of NEC, BPD, ROP, feeding tolerance, death, length of stay, neurocognitive development, breastfeeding rates at discharge, growth, and cost–benefit ratio.[6,17–19] Systematic reviews and meta-analyses

underline the methodological weaknesses of these studies (eg, most of them were not blinded to the intervention, some were sponsored by the formula companies) and their authors conclude that more studies are needed to evaluate the clinical outcomes precisely.[18]

Lower risk of necrotizing enterocolitis. NEC is the only clinical outcome for which we currently have solid scientific evidence in favor of DHM. Systematic reviews and meta-analyses show that formula feeding is associated with a higher risk of NEC when compared with DHM feeding.[6,18,19] There is also strong evidence showing an exclusive HM-based diet (ie, MOM or DHM, fortified with a human-derived, not bovine-derived, protein) is protective against NEC when compared with formula.[6,20–26]

The clinical evidence is supported by our developing understanding about the complex function of the intestinal barrier. NEC pathogenesis is multifactorial. Structural and functional immaturity of the intestinal barrier (characterized by increased permeability and an excessive immune or inflammatory response to exogenous stimuli), formula feeding, and dysbiosis (ie, alteration of the physiologic microbiota) are the most important factors in NEC development.[27–30]

The intestinal permeability in preterm infants is greater than that of term infants, and intestinal maturation depends on the type, initiation time, and advancement of the feedings in the first days of life.[31] Whereas exclusive breast milk feeding is associated with a faster maturation of the intestinal barrier,[32,33] formula feeding can damage the immature mucosa, increase the permeability, alter the microbiota, and upregulate the mucosal inflammatory responses, exposing the infant to an increased risk of NEC development.[33,34]

Availability of DHM for the preterm infants would offer them the possibility of avoiding feeding with formula containing bovine protein in their first delicate weeks of life.[6,35] In addition, access to DHM would result in earlier initiation and progress of enteral nutrition, and decrease the risk of proinflammatory effects deriving from fasting and prolonged parenteral nutrition.[25,35–39] DHM can have a direct protective effect on the intestinal mucosa thanks to its immunomodulatory bioactive factors, such as lysozyme, lactoferrin, oligosaccharides, secretory immunoglobulin A (IgA), essential fatty acids, antioxidant factors, and growth factors, even after pasteurization.

Feeding tolerance. The concerns about feeding intolerance and the risk of NEC development are the main obstacles for the initiation and advancement of the enteral nutrition in VLBW infants. Feeding intolerance has not been defined in uniformly in clinical studies, making it difficult to have solid scientific evidence on this matter. Until now, different surrogate markers such as duration of parenteral nutrition or time to reach full enteral feeding have been used. The studies conducted in 1980s[40–42] support the hypothesis that unfortified DHM feeding (as the sole diet or as a supplement when MOM is insufficient) improves feeding tolerance when compared with formula feeding in terms of decreased episodes of diarrhea, vomiting, gastric residuals, and suspension of the feeding. Other studies show that MOM and/or DHM feeding, even fortified, results in earlier initiation of enteral nutrition, faster increments of enteral milk volume, and decreased incidence of NEC.[21,22,25,43–53] A multinational survey[54] assessed the differences in feeding practices and concluded that the majority of the NICUs with access to DHM tend to initiate enteral feeding earlier, with a faster advancement when compared with NICUs without access to DHM.

Lower incidence of bronchopulmonary dysplasia. A few trials have evaluated BPD as an outcome measure. In 2005, Schanler et al[55] assessed BPD incidence as a secondary outcome measure in VLBW infants and observed a significant reduction of BPD incidence in the group fed fortified DHM vs preterm formula (15% vs 28%, respectively; $P = 0.048$). A metanalysis[56] evaluating 18 studies (n = 3 RCTs and 15 observational studies) reported that exclusive HM feeding (ie, MOM and/or DHM) led to a decreased incidence of BPD. DHM as the sole diet did not seem to be as protective as fresh MOM.

Retinopathy of prematurity. Two trials[44,57] and a systematic review[19] did not find any significant difference in ROP incidence between the groups fed fortified DHM or preterm formula.

Length of stay. The outcome for the hospital length of stay has been evaluated only by observational studies. In a retrospective study,[22] among 4 groups of VLBW infants fed differently, the group fed exclusively HM had a shorter length of stay relative to the other 3 groups (which were fed a bovine-based fortifier; a mixed combination of maternal milk, bovine fortifier and formula; and formula) ($P < 0.004$).

Mortality, neurodevelopment, and growth. In their meta-analysis, Quigley et al[18] did not report a statistically significant difference between infants fed DHM compared with formula in terms of the incidence of death (in the NICU and in the first 9 mo after discharge), and for growth and neurodevelopment during this time period.

Breastfeeding at discharge. Williams et al,[58] in their review of 10 nonrandomized studies, concluded that having access to DHM resulted in a 19% increase in

exclusive breastfeeding at discharge compared with the centers without access to DHM. This higher incidence of exclusive breastfeeding at discharge (although it did not reach a statistical significance in the meta-analysis) was in line with the Italian data published by Arslanoglu et al[59] and US data published by Kantorowska et al.[60] In both studies, the presence of an HMB on site or having access to an HMB had a remarkably positive impact for NICUs on the exclusive breastfeeding rates at discharge (Italian data: 60.4% vs 52.8%, $P < 0.04$; US data: 10% increase in breast milk feeding at NICU discharge).

Other clinical uses of donor human milk

Because of its limited availability, DHM is prioritized for VLBW infants in the NICU. But there are some other clinical conditions when DHM can be used and confer health benefits because of its nutritional and bioactive compounds, better tolerance by infants, and its positive impact on intestinal maturation.[5,61,62] These prioritized conditions for using DHM include gut priming, feeding of LBW infants, realimentation after gastrointestinal surgery (eg, intestinal resection, abdominal wall defects), growth failure due to intolerance to other foods, severe food allergies, metabolic diseases (particularly aminoacidopathies), immune deficiencies, chronic renal insufficiency, and cardiopathies.[5,7,18,33,42,61,63] Finally, it also has become an increasingly common practice to use DHM for term infants temporarily in the first days of life until the infant's mother builds a sufficient milk supply.[64,65] Temporary and long-term use of DHM as a natural supplement can have a much less negative impact on exclusive breastfeeding when compared with formula.[66–71]

ORGANIZATION AND QUALITY ASSURANCE

At the international level, there is a huge variety of regulatory approaches to HM banking. Although milk donation also carries risks, it is not subject to any legislation in many countries.[3,72,73]

Theoretical microbiological risks associated with DHM feeding are similar to those in the food industry and also to those associated with transfusion and transplantation.[73,74] The food industry's standard quality assurance tool is Hazard Analysis Critical Control Points (HACCP)[75] and it is widely recommended also for HM banking.

Most of the HMBs in various countries strictly follow the guidelines prepared by national scientific societies, which are based on the norms for blood donation[76] and have been adapted to the various risk levels deriving from the use of HM and on the norms for the food products,[77–79], with particular referral to HACCP principles and traceability and retraceability of the data.[80,81] In France, a state law regarding the regulation of milk banking has been issued, whereas in Italy, there are guidelines recommended officially by the Ministry of Health.[10–14,74,82–85]

Minimum requirements for the establishment of an HMB

There is no uniformity between various banks and there are no published universal guidelines defining the minimal requirements.[3] The following information has been extrapolated mainly from the guidelines from the Italian Ministry of Health.[74]

Location. An HMB should be functionally associated with at least 1 neonatology unit that is responsible for the care of preterm infants younger than 34 weeks.

Space. The space dedicated to an HMB should be structured in such a way that it permits an effective control of the activity and an thorough cleaning and disinfection.

Staff. Dedicated staff of the HMB should be quantitatively appropriate for the workload and should be skilled to handle all the activities of milk banking. The composition of the HMB staff (ie, doctors, nurses, technical and auxiliary staff) varies on the basis of the complexity of the service: responsibility, coordination, control of the procedures, recruitment and assistance of the donors, milk collection at home, processing and bacteriological testing of the milk, control and insurance of the hygiene of the materials used, recording of the medical and administrative documents, and distribution of the final product. HMBs may require consultations with various health disciplines, including microbiology, infectious diseases, neonatology, pediatrics, nutritional sciences, lactation consultants, and administrative support. Training of the HMB's staff is of utmost importance and should be documented.[74]

Devices. Minimal equipment requirements for HMBs include at least 1 pasteurizer, 1 freezer (kept at –20°C) with acoustic and visual alarms of temperature changes and thermoregistration, 1 refrigerator (kept at 0°C ± 4°C) with control of minimum and maximum temperatures[74]; a workstation and electrical milk pumps covering the needs of the donors[74]; and, if mono-use bottles are not being used, a dishwasher with thermodisinfection (+93°C for 10 min) and a system of hermetic closure of the bottles.[74]

Quality assurance and management

To ensure safe operation of an HMB and the safety and quality of the final product given to the recipient, an appropriately designed and implemented quality assurance plan should be in place.[3,10,14,73] Autocontrol and HACCP are essential for quality assurance[86] and include the following policies and procedures.

Autocontrol and hazard analysis and critical control points. The HACCP is a control system to ensure the safety of the food products; it provides a rational and organized way for autocontrol. Autocontrol implies the obligation of the operators at any level within the food chain to check the hygiene and safety of the product.[86] The system consists of a detailed analysis of each step of food production to identify and control potential hazards: biological, chemical, and physical. The concept, which was originally developed by the US National Aeronautics and Space Administration in the 1950s to guarantee the safety of alimentary products for the aerospace programs, has been adopted by the Parliament and Council of the European Union (CE) with directive 43/93/CEE, and then by the regulations CE 178/2002, and CE 852/2004.[77,78] The system has been in force in Italy since the beginning of 2006.[86]

Regulation CE 852/2004[78] encourages the use of correct operative and hygienic practice workbooks to implement the HACCP principles. Use of such a workbook, though not mandatory, is essential for the operators to follow the hygienic norms and prepare food safely.[86]

Identification of the hazards: definitions of critical control points and control points. For any phase of the productive process, it is necessary to evaluate the type (biological, chemical, or physical) and the levels of the risks for food safety. The scope of the HACCP prepared for an HMB should cover all the steps of milk banking starting from the donor selection to the distribution of the processed milk to the recipient.[77] The critical control points (CCPs) identify the phases of the productive process that are particularly critical, where the risk for food safety is detectable and is substantial in terms of the probability and gravity of the effect. It is necessary to perform a systemic control of actions for the CCPs: the risk is quantified with predefined numeric ranges, which permits monitoring of the productive phase. The monitoring procedures of CCPs and the corrective actions aim to eliminate the hazard or to reduce it to acceptable levels. The monitoring activity should be registered.

Control points are the phases of the productive process when the possible risks for food safety cannot be easily quantified but can be taken under control simply by the adoption of the preventive measures represented by good manufacturing practices (GMPs) and good hygienic practices (GHPs).[79]

Good manufacturing practices and good hygienic practices. GMPs indicate the operative conditions and requirements to guarantee the hygiene and safety in the entire food chain. They define the procedures to ensure the correct handling of the food and correct functioning of devices.[86] The GHPs define the modality and the frequency of the procedures performed for the hygiene of the staff and the cleaning and disinfection of the facility, devices, and the transport media of the milk.[86] Figure 1 shows a flow diagram representing the procedures of an HMB with identified CCPs, GMPs, and GHPs. Where applicable in the text, GMP and GHP are noted in parentheses.

Traceability and retraceability of the data. In the autocontrol manual, the procedures that permit traceability should be described. Traceability is a process of systematic registration of information related to the product during specific procedures of the food supply chain that can be done by with paper or computer support. Retraceability is the reverse process and makes it possible to follow the food process during the phases of production and distribution. Likewise, in HMBs, the labeling of all the milk containers is of fundamental importance. Ensuring the traceability of DHM from individual donation to recipient is crucial for the safety and quality assurance.[3,77] This is possible by keeping the records of all donors (ie, donor number, consent, questionnaire, blood test results) and their individual milk donations, the records of pasteurization batches (ie, a unique number, time and temperature of the pasteurization, microbial test results), the records of the pasteurized product to be given to the recipient, and finally, the records of the recipient (ie, parents' written consent for DHM, and the product number of each product the recipient receives).[3] All donor milk and containers should be labeled at each stage to ensure traceability and tracking of the milk. Traceability can be enhanced by customized and purpose-developed barcode tracking systems and the use of the internationally agreed-upon coding systems.[3,86]

LEGEND AND DEFINITIONS

In this section, we begin by describing the method for our evaluation using the GRADE classification. We then define specific terms used in this article.

Legend

Classification of the levels of evidence and formulation of the recommendations were made according to the

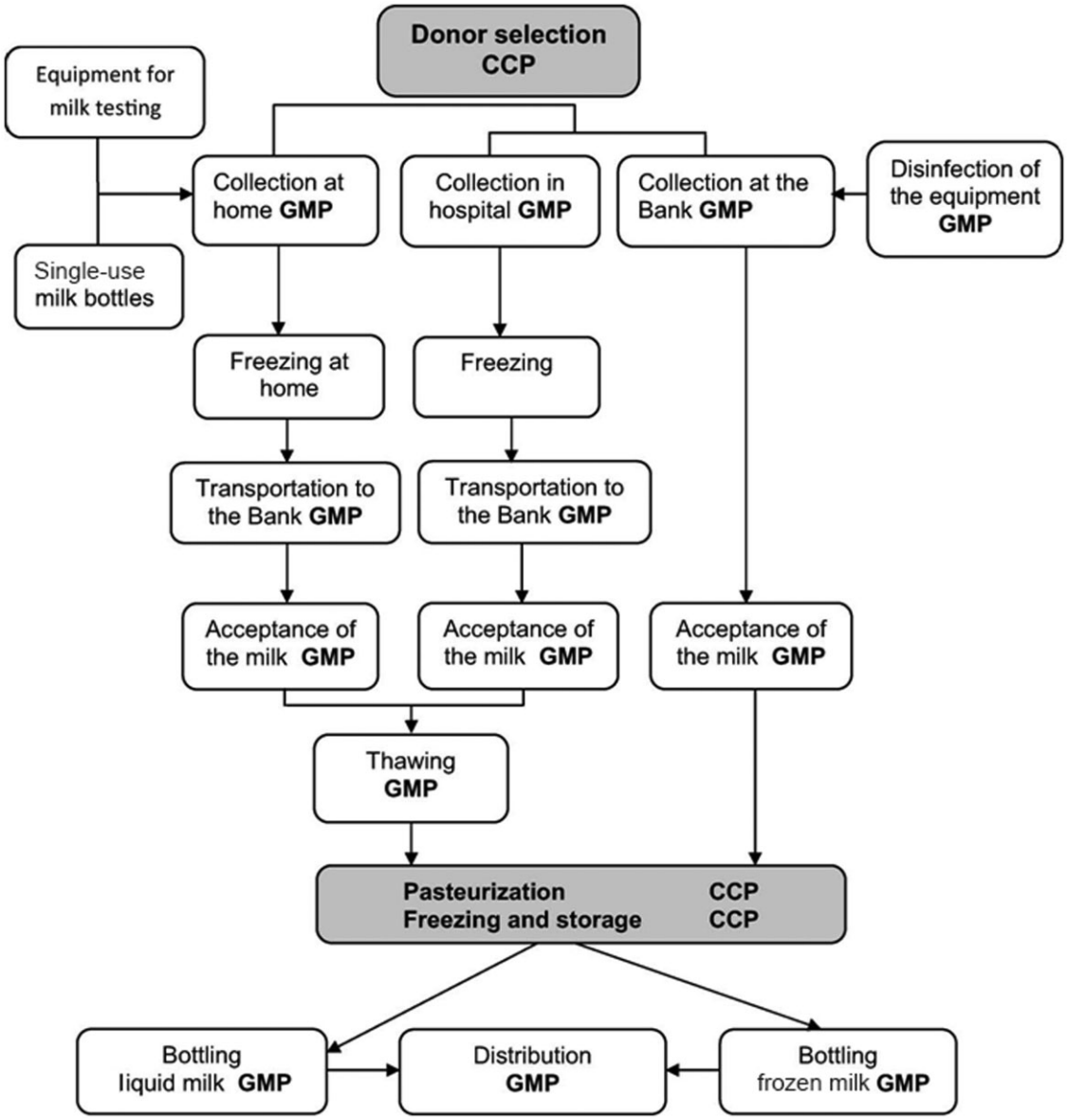

Figure 1 **Flow diagram representing the procedures of a human milk bank (HMB).** CCP, critical control point; GMP, good manufacturing practices.

GRADE classification.[71] This recommendation system is based on the categories of evidence that refer to clinical epidemiological studies. However, there are numerous conditions in the field of health care, many of which are dealt with in the recommendations presented in this article, for which clinical epidemiological studies are not available or not feasible, but biological, biochemical, pharmacological, or microbiological studies can be found.

We evaluated these studies considering their experimental design. Even in the absence of studies related to clinical application, indirect evidence can be highly relevant (eg, observation of significantly different bacterial counts in milk samples collected by different techniques or stored at different temperatures is relevant information, even if clinical studies on the effects of administration of these milk samples to the neonates are lacking).

Level of evidence

The quality of evidence is defined on the basis of the type of the study, as follows: high, indicated symbolically as ++++, means subsequent studies will hardly change the estimate of the effect; moderate (+++) indicates subsequent studies may have a significant impact on the estimate of the effect; low (++) indicates it is very likely that subsequent studies will change the estimate of the effect; and very low (+) means any estimate of the effect is very uncertain.

Grade of recommendation

The recommendation levels were categorized as follows: strongly in favor (indicated symbolically as ↑↑), adherence to the practice in question surely overcomes the undesirable effects. This practice is recommended. Weakly in favor (↑): adherence to the practice in question will possibly have more beneficial effects than the undesirable effects. This practice is suggested. Weakly against (↓): adherence to the practice in question has undesirable effects that will possibly exceed the beneficial effects. It is suggested not to apply this practice. And Strong against (↓↓): adherence to the practice in question will cause more undesirable effects than beneficial effects. It is recommended not to apply this practice.

GRADE classification further defines the recommendations, which are based on the experience of the Panel, where they are believed to be important, yet no scientific evidence seems to exist, as Good Practice Points.

Definitions

The following defined terms are presented to provide the foundation for this report.

- Donor human milk bank (DHMB): a facility established with the purpose of selecting, collecting, checking, processing, storing, and distributing DHM to be used for specific medical requirements.
- DHM: HM given voluntarily and freely to a DHMB.
- Raw (or fresh) human milk: HM that has not undergone heat treatment.
- Fresh, refrigerated human milk: HM stored at a constant temperature of +2 to +4°C.
- Fresh-frozen human milk: HM frozen and kept at a temperature no higher than –20°C.
- Pasteurized human milk: HM that has undergone a pasteurization process.
- Pooled human milk: a mixture of HM obtained from ≥1 donor(s).
- Preterm human milk: HM from women who delivered before the 37th week of gestation, collected within the fourth week after delivery.

OPERATING PROCEDURES

Donor selection and exclusion criteria

Specific criteria are needed to determine who can provide DHM. The criteria ensure that there will be enough milk available for donation and to minimize the risks inherent with HM donation.

Donor selection. Selection of donors requires an approach similar to that used for blood donors and should be done by a medical practitioner so that only healthy donors can be enrolled.[86–90] Donor selection aims to identify the specific conditions contraindicating the donation (permanently or temporarily), not only in the interest of the receiver but also of the donor herself and her own infant.

There is not a unanimous agreement on the eligibility of the milk donors, because there are different social and health realities in different parts of the world. But there is consensus that the screening should include the following steps[72]: (1) a precise anamnesis, including the donor's lifestyle, pathologic conditions, treatments, and tests performed during pregnancy as a routine for clinical problems; (2) physical examination; and (3) blood tests (of biological quality).

To be enrolled as donors, the candidate women should be in good health, should have a healthy lifestyle and negative blood test results, and should be producing milk in excess with respect to their own babies' needs. A written consent is required for the processing of the personal data, the blood tests, and the milk use.

In addition to accurate information on the milk extraction and storage, a constant relationship and dialogue between the HMB personnel and the donor are the main determinants to ensure the safety of the donated milk. Any kind of new event or behavior that can increase infectious or toxicologic risks has to be communicated immediately to the HMB. Blood tests of biological quality should be performed at the initiation of the donation. If the milk has been collected prior to the blood tests, it should not be used before the eligibility of the donor is confirmed. It is not necessary to repeat the blood tests during the donation period unless any change in the risk status occurs[13,73,81,85]; however, if the donation period lasts longer than 3 months, a new test is required.[11,82–84] It would be appropriate to follow a specific method to determine donor eligibility and provide adequate medical and psychological support for the mothers excluded from donation for any reason. Mothers who have lost their infants should not be excluded from donation if they are eligible and volunteer for the donation.[12,91–93]

One of the aspects that may cause reluctance to the acceptance of DHM by the parents of the recipient infant and some healthcare professionals is the potential risk of infection transmission.[94] Infections transmitted from the mother to her own infant through the milk are rare and have a much lower incidence than the transmission through blood.[95–98] When the procedures are performed correctly, the risk of contamination due to the use of DHM seems to be extremely low.[11,99]

Because there is not a specific national law for the donation of HM, we referred, while preparing this review, to the regulatory framework in force for the enrolment of blood donors[76] and to the reports of the Istituto Superiore di Sanità in Italy (from the methodological and epidemiological points of view).[100,101] Although there are many similarities between the donation of milk and blood, the level of risk accompanying the transfusion of blood derivatives vs feeding with donated milk is remarkably different.[1,97,98,102] Thus, when the scientific evidence permitted, specific criteria for the donation of HM, derogating from the rules of blood products donation, were indicated.

Exclusion criteria. Table 1 identifies how to evaluate lifestyle exclusion criteria for donors. The level of evidence is also provided for these criteria. The criteria are based on permanent and temporary exclusion criteria. The evidence for these criteria is provided next.

1.1 Lifestyle

Evidence for recommendations (1.1.1.–1.1.10.).

1.1.1. Nicotine and its main metabolite, cotinine, have been detected in the milk of mothers who smoke nicotine products, and it has been shown that nicotine levels in the milk of mothers who smoke were 1.5–3 times higher than those in plasma levels.[103] Negative effects have been reported in breastfed infants of mothers who smoke, such as poor weight gain, allergy (asthma), otitis media, upper and lower respiratory tract infections, leukemia, tumors, and metabolic syndrome.[4,12,104,105] Breast milk of mothers who smoke can have high levels of cadmium, copper, zinc, selenium, and magnesium, due to the combustion of tobacco.[4] Maternal smoking during lactation is associated with C and D hypovitaminosis, with alterations in the thyroid functions and immune responses due to the low levels of iodine, and reduced antiinflammatory and anti-infective cytokine levels, respectively, and with a reduced breast milk content of n-3 long-chain polyunsaturated fatty acids because of decreased lipoprotein lipase activity.[4,107] In addition, nicotine has considerable negative effects on the infant's central and peripheral nervous systems, including altered sleep and wake patterns, irritability, and reduced heart rate.[4]

The American Academy of Pediatrics[4] does not consider smoking a contraindication for breastfeeding. For DHM, it has been decided to apply more rigid criteria; therefore, women who smoke are prudentially excluded from donation.[11,12]

1.1.2. The American Academy of Pediatrics, in its most recent document based on literature revision,[106] strongly recommends that nursing mothers do not take any drugs of abuse (eg, amphetamine, cocaine, heroin, marijuana), because of the potential harmful effects these can have on their infant. Marijuana use can affect the behavior of the neonate, including a tendency to lethargy, difficulty in nutritive sucking, and delay in neurobehavioral development.[4,106–113] Cocaine use can result in toxicity in the neonate, presenting as seizures, irritability, vomiting, diarrhea, and tremors.[106,110,114–116] Maternal benzodiazepine use can also have harmful effects on the infant's central nervous system; its long half-life results in the

Table 1 **Lifestyle exclusion criteria for donors with level of evidence**

Recommendations for exclusion	Quality of evidence	Strength of recommendation
Permanent exclusion		
Potential donors who:		
1.1.1. Smoke, or who are exposed to passive smoking, or use nicotine-containing products (including electronic cigarettes, patches, etc.)	+++	↑↑
1.1.2. Use marijuana, cocaine, amphetamine, benzodiazepine, or other drugs of abuse	++++	↑↑
1.1.3. Consume daily xanthine >300 mg (eg, coffee, tea, cola, cacao) or energy drinks	+++	↑
1.1.4. Follow a vegan diet without vitamin B_{12} supplementation	+++	↑
1.1.5. Consume daily alcohol in a quantity greater than the CDC recommendations	++++	↑↑
Temporary exclusion		
Potential donors who:		
1.1.6. Have/had sexual intercourses with partner(s) who is (are) HBV, HCV, or HIV positive, or who is (are) at high risk (eg, sexual behavior, drug use) of acquiring serious bloodborne infections	++++	↑↑
1.1.7. Have close contacts with HBV carriers (eg, sharing the same house or close relationship)	++++	↑↑
1.1.8. Had piercing, tattooing if not performed with a disposable single-use needles*	++++	↑↑
Permanent or temporary exclusion		
1.1.9. Potential donors from countries where particular infective agents are endemic. In this case, specific tests are performed to decide for either permanent or temporary exclusion	++++	↑↑
Exclusion on case-by-case basis		
1.1.10. Potential donors who are exposed to pollutants	++	↑

*See Recommendations 1.4.

Abbreviations: CDC, Centers for Disease Control and Prevention; HBV, hepatitis B virus; HCV, hepatitis C virus.

accumulation of toxic metabolites in the neonate's plasma.[106] To be on the safe side, mothers who use other drugs, for which there is not enough scientific evidence about risks of exposure in HM, also are excluded from donation.[11,12]

1.1.3. Maternal xanthine consumption of >300 mg/day can cause irritability and sleep disturbances in the breastfed infant and reduces the concentration of bioavailable iron. Caffeine clearance in breastfed infants can be <10% of that of adults (a half-life of 97 h in infants vs 5 h in adults).[117] A cup of domestic coffee or espresso contains 80–90 mg of caffeine, whereas caffeine content of a cup of tea is 20–30 mg. A 330- mL cola has 40 mg of caffeine, and 100 g of dark chocolate has 70 mg.[4,12,106,118–120] Energy drinks containing ginseng, ginkgo biloba, and yerba mate also can have stimulating effects on the neonate, leading to sleep disturbances, irritability, and agitation.[119,121]

1.1.4. A strict vegan diet causes vitamin B_{12} deficiency and megaloblastic anemia with neurologic abnormalities in the infant.[12,80,122–125]

1.1.5. Because there is no specific evidence on safe amounts of alcohol consumption during lactation, these recommendations refer to the US Centers for Disease Control and Prevention guidelines that daily alcohol consumption by lactating mothers should not exceed 45 mL of strong alcoholic beverages, 360 mL of beer, or 150 mL of wine. Alcohol can have harmful effects on the neonate, including neuromotor developmental alterations, postnatal growth restriction, and sleep disturbances.[4,11,12,80,126,127]

1.1.6.–1.1.8. Occasional sexual relations, living with people positive for hepatitis B virus (HBV) or hepatitis C virus (HCV) (either sexual partners or nonsexual partners), intravenous drug use, and tattoos are the major risk factors for hepatitis B and C contamination. Casual sexual intercourse also is a risk factor for AIDS.[100,101]

1.1.9. A prospective donor and her sexual partner occasionally can undergo some serological tests for specific diseases with high prevalence in the zone where the donor lives (eg, women positive for human T-cell lymphotropic virus (HTLV) I/II or *Trypanosoma cruzi* are excluded from donation).[73,85,89]

1.1.10. There are no Italian data regarding environmental pollutants, but even in the conditions of documented pollution, breast milk is preferable to infant formula.[72–75]

1.2. Maternal diseases and pathological conditions

In addition to lifestyle criteria, there are several maternal diseases and pathological conditions that lead to the exclusion of donors, as presented in Table 2. The evidence for the infectious and noninfectious criteria for the protection of babies and donors is provided next.

Evidence for recommendations (1.2.1.–1.2.2.).

1.2.1. Not all the pathogens present in the blood of a lactating woman with a systemic infection can be isolated from her breast milk, and the microorganisms found in breast milk do not always infect the infant and cause adverse effects. For some of maternal infections, such as those caused by HIV, HTLV, Lassa virus, arenavirus, yellow fever virus, members of the Filoviridae (eg, Marburg virus, Ebola virus), and *T. cruzi*, breastfeeding is not recommended. Although the milk of mothers infected with HBV and HCV not associated with HIV contains viral genome, it is not considered as a certain vehicle of infection (unlike blood). There is an increased risk of transmission when rhagades are present.[4,102,128–130]

Breast milk is not an important vehicle of infection by the respiratory transmitted viruses.[102] SARS-CoV-2, like the other coronaviruses, is inactivated by Holder pasteurization.[131–133] Cytomegalovirus (CMV) can be a problem for preterm infants with gestational age <32 weeks, who are immunologically depressed, if the milk does not undergo a heat treatment that inactivates the virus.[134,135] Papillomavirus does not seem to be transmissible via milk; the transmission of West Nile virus appears to be rare and adverse effects have not been shown; for Zika virus, no infections have been reported in breastfed infants.[97,136] Transmission of malaria through breast milk has not been documented,[102] and syphilis[137] and tuberculosis[138] do not seem to be transmitted through breast milk (breastfeeding is contraindicated only if there are mammary lesions).

The transmission risk of the prions (responsible for Creutzfeldt-Jacob disease) is unknown.[12]

Most viral and bacterial infectious agents are transmitted in HM. However, almost all can be inactivated by pasteurization with the Holder method (62.5°C for 30 min).[139] Microbiological safety of the milk is increased further by the systematic bacteriological analysis of the milk (see Recommendations 3.1.). Data on the impact of Holder pasteurization on the infectivity of hepatitis B and C viruses are still lacking. The only data available show inactivation of HCV following heat treatment at 60°C for 10 hours.[140,141] Although there is uncertainty about the transmission of these viruses through the milk, it is appropriate to exclude the mothers from donation who are serologically positive for HBV and HCV.

It is clear that Holder pasteurization inactivates HIV[142]; prudentially, however, it is appropriate to exclude HIV-positive women from donation. Holder

Table 2 **Exclusion criteria for maternal diseases and pathological conditions**

Recommendations for exclusion	Quality of evidence	Strength of recommendation
1.2.1. Infectious diseases		
Potential donors who:	++++	↑↑
a. Are infected by HBV, HCV, HIV, *Treponema pallidum* or are tested positive for 1 of these infections: Permanent exclusion		
b. Are infected by other infections (eg, viral, bacterial, protozoal) should be evaluated case by case on the basis of anamnesis, specific clinical conditions, and recovery Permanent or temporary exclusion		
c. Are seropositive for CMV: not excluded from donation		
d. Had contacts with patients with infectious diseases (eg, varicella, mumps, rubella): If the donor is not immune, temporary exclusion during the incubation period; if the immunity status is unknown, temporary exclusion for 4 wk after the final contact		
e. Have mastitis: Temporary exclusion until 24 h after the completion of the antibiotic therapy, which resulted in a complete recovery		
f. Have Creutzfeldt-Jakob disease: Permanent exclusion		
g. Have tuberculosis: Temporary exclusion until a complete recovery		
h. Have mycosis of the nipple, reactivation of HSV or varicella zoster infection in the mammary or thoracic region: Temporary exclusion as long as the skin lesions exist		
1.2.2. Other diseases		
Potential donors who:	++	↑
a. Have tumors (except basocellular cutaneous carcinoma and in situ cervix carcinoma after the removal)		
b. Have autoimmune diseases not limited to a single organ		
c. Have other diseases in active, chronic, relapsing, disabling, or weakening forms		
These conditions do not represent contraindications for breastfeeding, but as a principle, we suggest excluding these women permanently from donation to protect their health.		

Abbreviations: CDC, Centers for Disease Control and Prevention; CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; HSV, herpes simplex virus.

pasteurization also inactivates HTLV-I and HTLV-II[139]; the scarce occurrence in Italy[143] does not justify screening of the donors in Italy, unlike screening for HIV. CMV, like many other viruses, is very sensitive to the heat and thus is inactivated completely after Holder pasteurization.[144,145] The heat treatment methods that preserve the biologically active factors of the milk inactivate CMV completely. An example is pasteurization at 72°C for 5–15 seconds[144,146]; recent studies show that heat treatment at 62°C even for only 5 seconds, with instruments enabling processing of small volumes of milk, results in complete inactivation of the virus.[147–149] Freezing at –20°C, which was recommended in the past to protect preterm infants against CMV infection, does not eliminate the virus completely and does not seem to be safe.[150,151]

Holder pasteurization inactivates *Mycobacterium tuberculosis*.[139] It has not been demonstrated that breastfeeding is associated with transmission of syphilis[137] or tuberculosis[138] unless there are lesions of the breasts. However, prudentially, it is considered appropriate to avoid milk donation from mothers with active infection, in agreement with the Human Milk Banking Association of North America guidelines.[12]

1.2.2. Exclusion is indicated mainly for the protection of the prospective donor's health and because of pharmacological therapy that is often incompatible with the donation. The Human Milk Banking Association of North America guidelines[12] recommend a permanent exclusion of donors with leukemia and lymphoma, and a temporary exclusion of 3 years for other tumors undergoing treatment. There are conflicting data regarding the transmission risk of antiplatelet antibodies into the milk of women with autoimmune thrombocytopenia.[152,153]

1.3. Maternal therapies and diagnostic procedures

There are also maternal therapies and procedures that lead to excluding donors from providing HM. These permanent and temporary exclusion criteria are presented in Table 3. The evidence for these recommendations is provided next.

Evidence for recommendations 1.3.1.–1.3.8.

1.3.1.–1.3.5. The risk for donors who have undergone the aforementioned diagnostic and therapeutic procedures is related to the potential transmission of infectious agents, particularly HBV and HCV.[100,101]

Table 3 **Exclusion criteria for maternal therapies and diagnostic procedures**

Recommendations for exclusion	Quality of evidence	Strength of recommendation
Potential donors who:		
1.3.1. Had cornea or dura mater transplants (for spongiform encephalopathy risk): Permanent exclusion	+++	↑↑
Had other organ, tissue, cell transplants of human origin: Temporary exclusion*		
1.3.2. Had major surgery, diagnostic, or therapeutic interventions (eg, endoscopy, or interventions with flexible instruments such as bronchoscopy or colonoscopy, catheterization, and acupuncture if not performed with a single-use needle and by professionals): Temporary exclusion*	+++	↑↑
1.3.3. Received blood or blood-derived transfusions, plasma-derived products (including anti-D immunoglobulins, in utero transfusions), hemodialysis: Temporary exclusion*	+++	↑↑
1.3.4. Had minor surgical interventions (including dental procedures such as extraction, devitalization, and similar treatments): Temporary exclusion for a week*	+++	↑↑
1.3.5. Had vaccination:	+++	↑↑
Temporary exclusion for 4 wk after vaccination with an attenuated live virus.		
Temporary exclusion for 48 h for other vaccines		
1.3.6. Had pharmacological therapy: the use of drugs or pharmacologically active substances should be evaluated on a case-by-case basis.	+++	↑↑
The use of the following drugs does not require exclusion:		
• Replacement hormones (insulin, thyroid hormones)		
• low-dose progestogens and estrogen progestins		
• nonsedating antihistamines		
• hydrocortisone		
• inhalation drugs for asthma		
• nasal sprays		
• eye drops		
• topical treatments (if applied on mammary skin, breast should be cleaned before milk extraction)		
• substances administered orally and not absorbed		
• supplements of vitamins, minerals, oligoelements, essential fatty acids, probiotics		
The use of other drugs can require a variable exclusion duration: *Temporary or permanent exclusion*		
1.3.7. Received contrast material: should be evaluated on case-by-case basis	++	↑
1.3.8. Take dietary supplements or herbal products, including galagtogogues, without any specific medical indication: should be evaluated on case-by-case basis	++	↑

*See Recommendations 1.4.

Abbreviations: CDC, Centers for Disease Control and Prevention; CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; HSV, herpes simplex virus.

The risk of infection via transfusion of blood products has been reduced remarkably in the past few decades.[101]

In terms of the vaccines containing noninfectious viral genetic material carried by inactive, nonreplicating viruses (as in the example of SARS-CoV-2), it is recommended that donors are excluded from donation for 48 hours.[154]

1.3.6. Most of the drugs used by mothers during lactation are secreted into the milk. The concentration and the potential toxicity vary substantially depending on the substance, the dose,[155] and the clinical conditions of the infant (eg, prematurity, maturation level of the renal and hepatic functions and of the blood-brain barrier, plasma protein levels, contemporary use of other drugs). Only cytotoxic and chemotherapeutic drugs are contraindicated for the healthy infant.[4,11,12,106,119,127] It should be noted that the recommendations refer to breastfeeding, and more rigorous criteria must be applied to ensure the safety of DHM, which is being used mainly for vulnerable sick infants. The donors should contact HMB personnel when they start to take any kind of medication. Some drugs do not require any exclusion from donation,[12] other drugs considered compatible for breastfeeding (ie, those with no or negligible adverse effects) should be evaluated on case-by case basis considering their active ingredients and pharmacokinetics.

1.3.7. The recommendations regarding breastfeeding by mothers undergoing diagnostic procedures with contrast material differ from depending on the product used.[12,106,156] In the case of donation of HM, it is recommended to refer to the cited documents.

1.3.8. Dietary supplements and herbal products, including substances labeled as galactagogues, can contain potentially toxic substances uncontrolled in terms of either quantity or origin.[4,105,121,123]

1.4. Biological qualification tests

The biological qualification tests presented in Table 4 that are performed through blood withdrawal at the initiation of the donation contribute in an important way to identify pathologies in the preclinical phase. The evidence for these tests and how to apply them to ensure a safe supply of HM are presented next.

Evidence for recommendations 1.4.1–1.4.3

1.4.1. This recommendation is based on the fact that, for blood and blood products donation, the Italian epidemiologic situation, although changed in the past few years, does not require tests for emerging viruses among the obligatory tests.[89]

1.4.2. This recommendation is consistent with other guidelines: most guidelines, relative to the local epidemiological characteristics, include also HTLV-I/II serology.[11,12,82,84,151] The waiting period to perform laboratory tests after an event putting the potential donor at risk is based on the fact that the serological tests (particularly for HCV) can become positive later (ie, window period).

1.4.3. In Italy, the biological qualification tests performed legally for blood donation[76] are a combination of serological markers (anti–HIV-1 and -2, HIV antigen, anti-HBV, anti-HCV, Venereal Disease Research Laboratory test, and *Treponema pallidum* hemagglutination test) and the viral genomes of HBV and HCV, and the HIV nucleic acid test. This association reduces the risk of enrolling infected donors, and shortens the window period when compared with serological tests,[101] and allows early diagnosis of an asymptomatic infection, thus providing protection also for the donor.

In the case of milk donation, shortening of the window period avoids permanent exclusion of the prospective donors who report an event with a risk of infection in the perinatal period. Breast milk can be expressed during the phase of temporary exclusion and kept in quarantine until the tests are performed, and the milk can be used if the tests are negative (which is what is happening in almost all cases).

The adoption of nucleic acid testing requires a more complex organization and relatively higher costs when compared with the use of serological tests only. On the basis of these considerations, and in line with the literature, it is recommended as the gold standard, at least for the women who report recent risk factors, to adopt the aforementioned protocol used in the blood banks.[81,85] The actual regulatory norms for blood donation indicate that the tests should not be performed before 4 months after the event or condition with high risk for infection[76]; however, because the scientific studies suggest a shorter duration would be sufficient,[98,158–162] it is recommended waiting for 2 months after the risky event and then running tests. This 2-month period has been indicated prudentially, and it is longer than the shortened window period ensured by the combination of the nucleic acid testing with the serological tests. With this combination of tests, even the longest window period, that for HBV, is 1 month.[159]

2. Collection and storage of the milk

HM is a medium in which infectious agents can multiply very rapidly. For this reason, collection and storage of the milk must be done based on the HACCP principles, following carefully hygienic norms to avoid contamination and having to discard the milk. The collection and storage of the milk can be done either at the HMB or at the donor's home; in the latter case, the equipment necessary for the expression of the milk is provided by the HMB, including bottles and a milk pump (a manual pump is sufficient). At the bank, the HMB operators should apply the norms defined in the autocontrol manual to ensure personal hygiene and sanitation of the equipment, devices, and the working environment (GHP). HMB operators must teach the donors not only the correct way to collect and store the milk (GMP), but also the importance of the sanitation of the milk pumps, refrigerators, work surface, and so forth, if the procedures are being performed at home. The support provided the donors should also include the information on how to maintain adequate production and how to manage the milk production in excess.

2.1. Milk expression

The collection of milk should also be done under standardized conditions following specific recommendations, as presented in Table 5. The evidence for these recommendations is provided next.

Evidence for recommendations 2.1.1–2.1.5

2.1.1. Before milk expression, it is necessary to sanitize the work surface, remove rings and other jewelry, and wash the hands thoroughly with running water and soap for a minimum of 60 seconds, paying special attention to the spaces between the fingers and periungual areas. Scientific evidence based on meta-analyses of RCTs and an intervention study demonstrate that thorough handwashing significantly reduces the transmission risk of hospital infections.[163–165] Thorough

Table 4 **Exclusion criteria based on biological qualification tests**

Recommendations for exclusion	Quality of evidence	Strength of recommendation
1.4.1. It is recommended that all potential donors undergo serological tests, prior to the initiation of donation, to exclude positivity for HIV, HBV, HCV, and syphilis infections. Even if only 1 of the tests is positive: Permanent exclusion	++++	↑↑
1.4.2. The following serological tests are the norm: anti-HIV 1/2, anti-HCV, anti-HBc, HBsAg, VDRL, and TPHA. If any condition for the risk of infection transmission is reported, serological tests should not be performed before 6 months after that event/condition.	+++	↑↑
1.4.3. The combination of molecular tests for HIV, HBV, HCV, and the serological tests (anti-HIV 1 and 2, HIV antigen, anti-HCV, HBsAg, VDRL, and TPHA) is the gold standard and represents the norm for screening of the blood donors. Yet, it also offers advantages for the selection of milk donors. It is recommended that the potential donors reveal recent conditions creating risk for infection transmission. In this case, these tests should not be performed before 2 months after that event/condition.	+++	↑↑

Abbreviations: HBc, total hepatitis core antibody; HBsAg, hepatitis B surface antigen; TPHA, *Treponema pallidum* hemagglutination; VDRL, Venereal Disease Research Laboratory (test).

Table 5 **Recommendations for milk expression**

Recommendation	Quality of evidence	Strength of recommendation
2.1.1. Before each collection, it is of fundamental importance to wash the hands accurately.	++++	↑↑
2.1.2. The breasts, particularly the areolar zone and the nipples, should be cleaned very well. To avoid harming the skin with frequent use of detergents, it is sufficient to wash only with running water or with gauze soaked in water.	++	↑
2.1.3. There is no need to discard the first 5–10 mL of expressed milk with the aim of reducing the bacterial load.	++	↑
2.1.4. It is possible to empty the breasts through hand expression and through manual or electric breast pump. An electric pump seems to be a more practical and efficient instrument for a complete emptying of the breast and for prolonged donations. It is preferable to use the models that simulate the dynamics of the infant's suction cycle and that include the equipment for simultaneous pumping of both breasts.	++++	↑↑
2.1.5. It is essential to follow carefully the hygienic norms in any setting, particularly when the milk is collected at home. Special care should be taken to clean and disinfect all the components of the breast pumps.	++++	↑↑

handwashing reduces the contamination risk of the expressed milk.[165]

2.1.2. Daily personal hygiene is important. Washing the breast with disinfectant solutions has not been shown to be more effective than washing the breast with water alone.[166]

2.1.3. The study results of West et al,[167] which pointed out the possibility of greater bacterial contamination in the first 10 mL of extracted milk, have not been confirmed by 3 successive studies of a better experimental quality.[168–170]

2.1.4. Use of electric breast pumps, with respect to hand expression, results in the collection of a greater volume of milk,[171–173] particularly if milk is expressed from both breasts simultaneously.[174,175] Electric breast pumps that simulate the suction dynamics of the baby are the most efficient,[175,176] particularly models with 2 distinct phases: the first phase is so- called stimulation of the milk ejection reflex, the second phase is the expression phase.[176,177]

2.1.5. Three studies,[178–180] 1 of which was an RCT,[180] demonstrated that hand expression of breast milk at home reduces the risk of bacterial contamination when compared with the other methods. Use of electric breast pumps requires careful observation of the hygienic norms to reduce the risk of bacterial contamination.[181,182]

In the case of milk collection in the hospital, no significant difference concerning the contamination rates has been observed among the expression methods.[170,180]

2.2. Washing and disinfection of the equipment

In addition to personal hygiene for the collection of DHM, it is necessary that Banks provide standards and recommendations for the equipment used for milk collection. These recommendations are presented in Table 6. The evidence for these recommendations is provided next.

Table 6 **Standards and recommendations for the equipment**

Recommendation	Quality of evidence	Strength of recommendation
2.2.1 All the materials that have been in contact with the milk should be washed with hot water and soap, and rinsed thoroughly after use to eliminate all organic residuals.	++++	↑↑
2.2.2. Appropriate cleaning and disinfection of the breast pump equipment that has been in contact with the milk are necessary. The equipment should be cleaned and sanitized with a gauze moistened with disinfectant before and after each use.	+++	↑↑
2.2.3. In HMBs where glass bottles are used, the use of a bottle washer with thermodisinfection is recommended as an alternative to a classic bottle washer with a brush, and bottles should be autoclaved for sterilization. Also, a washing machine used exclusively for this purpose and that fulfills the thermodisinfection conditions is acceptable.	+++	↑↑
2.2.4. The HMB staff is recommended to follow personal hygiene norms and regularly perform decontamination of the equipment and sanitization of the environment at the HMB to ensure the safety of the product. The same recommendations apply for the extraction and the storage of the milk at home.	++++	↑↑

Abbreviation: HMB, human milk bank.

Evidence for recommendations 2.2.1.–2.2.4

2.2.1. According to the indications of the US Centers for Disease Control and Prevention and of the European Parliament Regulation (CE) N. 852/2004,[78] washing with hot water and detergents is sufficient to ensure the hygiene of the material that has been in contact with food, because it removes the dirt and the microorganisms, resulting in a reduction in the microbial load. For hygienic safety (GHP), it is always necessary to clean the material in use and remove organic residuals (which can reduce the activity of the disinfectants) before the disinfection process (cold or hot disinfection) or to sterilization.[183–185] All methods have some disadvantages or risks.[189,190]

2.2.2. The external parts of the milk pumps (either manual or electric pumps) should be cleaned and disinfected with proper products before and after their use (particularly if each device has been used by more than 1 donor at the HMB), because breast pumps can be potential sources of contamination.[181,182,188]

There is no evidence supporting the necessity for sterilization of the breast milk pump components that come in contact with the milk after their use.[189] Sterilization after each expression can be justified if the same device is being used by more than 1 donor. In any case, it is essential to respect the indications of decontamination provided by the manufacturer. There is scientific evidence for the hygienic safety of so-called hot and cold disinfection.[86]

2.2.3. Washing carried out at a temperature of 93°C for 10 minutes (ie, thermodisinfection) has fungicidal and bactericidal effects and results in viral inactivation.[86]

2.2.4. Decontamination of the equipment and sanitization of the environment of the HMB, based on the plan of autocontrol (GHP), plays an important role for the safe handling of the milk and should be documented. At-home milk collection organized by the HMB staff offers an opportunity to verify the hygienic conditions in which expression and storage of the milk occur (GHP).

2.3. Milk containers

Currently, glass and hard plastic (ie, polycarbonate, polypropylene, polyethylene) or soft plastic (ie, polyethylene-vinylethylene) containers are available on the market. Table 7 lists recommendations and the level of evidence for the containers that are used to store DHM. The evidence for these recommendations is next.

Evidence for recommendations 2.3.1.–2.3.2

2.3.1. No relevant differences have been observed in the stability of some constituents of HM (namely, leucocytes, total immunoglobulin, IgAs and water-soluble constituents) stored in glass or in rigid plastic (polypropylene) containers.[190–192] Currently, the rigid plastic containers are being used more than the glass containers, because the latter are considered to have a potential risk for operators (wounds due to cutting) as well as for infants (microparticles of glass in the milk). The plastic containers contribute to environmental pollution. Polycarbonate can release bisphenol A,[193,194] and for this reason, they should not be used.[195]

2.3.2. Soft polyethylene bags reduce some components of HM (ie, significant loss of lipids and fat-soluble

Table 7 **Recommendations for milk containers**

Recommendation	Quality of evidence	Strength of recommendation
2.3.1 Glass containers and single-use hard plastic containers may be used.	++++	↑↑
2.3.2. Soft polyethylene bags are not recommended.	+++	↑↑

vitamins has been reported).[196] They are also difficult to seal and easily contaminated and broken.

2.4. Milk storage at home

The inevitable manipulations to which HM collected at home is exposed bring the risk of contamination and loss of milk physicochemical stability and biological activity. Thus, it is very important that, after its expression, the milk to be donated is handled and stored in the best possible way. Table 8 provides recommendations for milk collection and storage at home.

The donor must always write on each bottle her identification code and the collection date so, when necessary, she can be identified. The HMB should be informed when the donor takes any kind of medication.

Evidence for recommendations 2.4.1.–2.4.5

2.4.1. The germs in HM multiply very rapidly at room temperature.[197–199] Exposure to a temperature higher than 15°C can also cause lipolysis of fatty acids in the milk.[197]

2.4.2. At +2 to +4°C the bacterial growth in fresh HM is reduced and bacterial count does not increase significantly before 24 hours.[199–201] The bactericidal activity of fresh HM persists for 48 hours in the refrigerator and decreases significantly only after storage for 72 hours.[202] The reduction in the bactericidal activity of the refrigerated HM is compensated for by the increased bacterial capture by the fat-globule membranes.[203] Several studies did not show any significant bacterial growth during the milk storage in the refrigerator for periods longer than 24 hours.[165,204,205] Other studies indicated that fresh HM can be stored in the refrigerator for longer periods, from 4[206] to 8[207] days.

Despite the evidence, we believe it is unnecessary to prolong the refrigeration period. Regarding milk collection at home, because optimal hygienic conditions cannot always be ensured, we advise that HM be frozen within 24 hours after its expression.

The addition of fresh milk to the frozen milk can result in defreezing, with increased hydrolysis of triglycerides,[208] and can cause difficulty in identifying the collection date.

2.4.3. Freezing increases the milk volume in the container.

2.5. Milk transportation

Transporting milk to the HMB is another area that needs to be standardized and should follow hygienic procedures. The application of the hygienic safety system based on the HACCP principles is necessary.

Transport can be managed by the donor herself, by a service organized by the HMB (with a dedicated staff), or by third parties who assume this responsibility for the bank. It is preferable that the bank itself organizes the collection and transportation of the donated milk from donor's home to the HMB because doing so ensures the standardization of the procedures and also offers an opportunity to verify the conditions in which the collection and storage of the milk are done, which may help solve potential problems and strengthen the relationship and the dialogue with the donor. Recommendations for these procedures are presented in Table 9. The evidence for these recommendations are given next.

Evidence for recommendations 2.5.1.–2.5.2

2.5.1. Recommendations are in line with the Directive of Legislation (CE) N. 852/2004 of the European Parliament and Council[77] on the hygiene of dietary products, adopted in Italy on January 1, 2006.

2.5.2. Milk freezes at a lower temperature than water. The use of common ice can result in a partial melting of the milk during the transportation.

PROCEDURES FOR THE MANAGEMENT OF MILK AT THE BANK

The donated milk, when it has arrived at the bank, should be checked, pasteurized, stored, and distributed, as specified follows. At arrival of the donated milk at the HMB, the transportation and packaging conditions, the labels, the milk sample conditions (ie, frozen or not frozen), and the transport documents should be checked and registered. All the activities should be performed in line with specific operative procedures and registered on an appropriate form of the autocontrol plan.[86]

Breast milk, even in the absence of pathological conditions, contains microorganisms from the skin of the breast or other sources. Most of these are commensal microorganisms and symbiotes (ie, bacteria and fungi that represent a component of the microbiota of the infant); however, the pathogenic microorganisms can be isolated from HM. The microorganisms, besides being a risk for infection in some cases, are also

Table 8 **Recommendations for milk collection and storage at home**

Recommendation	Quality of evidence	Strength of recommendation
2.4.1. The milk collected at home and destined to be donated must remain at room temperature for the shortest possible time. After each milk collection, the milk container must be sealed and cooled immediately under running water.	+++	↑↑
2.4.2. While waiting for the transportation of milk to the HMB, 2 methods can be used for the storage: a. Immediate refrigeration followed by freezing (the method to be used when there will be additions to the collected milk): i. Freshly expressed and cooled milk should be put in the refrigerator at +2 to +4°C. Until the container is nearly full, it is possible to add the milk collected by subsequent extractions. Between collections, the container should be maintained in the coldest area of the refrigerator, distant from its door, and isolated from the other food. ii. The container with the refrigerated milk should be transferred to the freezer at a temperature not warmer than −20°C within a period ≤24 h after the first extraction. If the refrigerator does not have a reliable temperature-monitoring system (generally most of the domestic refrigerators do not have this system), it is recommended to keep the milk in the refrigerator for no longer than 12 h. b. Immediate freezing: After extraction, if more milk additions are not planned, the container is placed directly in the freezer. It is not recommended to add freshly expressed milk to the frozen milk.	+++	↑↑
2.4.3. The container to be frozen should never be filled completely. It is recommended, for example, that a 250-mL bottle should be filled with a maximum of 200 mL of milk.	+++	↑↑
2.4.4. For the recommended maximum storage period, please see Recommendation 3.3.	+++	↑↑
2.4.5. In domestic refrigerators, to minimize the risk of contamination, it is recommended to store the milk bottles in containers that isolate the milk from the other food inside a regularly sanitized refrigerator.	++	↑

Table 9 **Recommendations for milk transportation**

Recommendation	Quality of evidence	Strength of recommendation
2.5.1. The transportation of the milk collected at home should be carried out respecting the "cold chain', in such a way that the milk arrives at the HMB in a frozen state. For greater safety, it is preferable that the HMB itself assumes the responsibility of the collection and transportation of the milk from donor's home to the HMB. Transport freezers, thermal bags with dry ice, or refrigerating packs can be used.	+++	↑↑
2.5.2. Avoid the use of common ice.	+++	↑↑

Abbreviation: HMB, human milk bank.

responsible for the deterioration of the physicochemical characteristics of the milk.

Fresh MOM, expressed in the hospital and stored appropriately, does not require routine culturing or heat treatment.[95,97,209–211] Although it contains microorganisms, thanks to its numerous bioactive antimicrobial components, MOM can rarely be a source of infection. Because DHM is generally being used for vulnerable infants and has fewer anti-infective properties than fresh HM, because of the heat treatment, it should undergo bacteriological analysis that measures the quantity of the bacteria in the milk. If the milk is contaminated beyond a certain limit, it should be discarded because it does not fulfill appropriate safety and quality requirements. A high bacterial count can be caused by factors associated with the expression, storage, transport, and handling (pre- or post-pasteurization) of the milk, rather than by any important health problem of the donor. Pasteurization is an effective tool for the sanitization of the milk,[212] but it might not eliminate all pathogenic microorganisms, especially when the bacterial load is high.[213] For these reasons, it is essential that the HMB staff, adequately trained, regularly verify reliability of the donors and procedures, and suitability of the environment and the devices, based on HACCP principles, via routine bacteriological and quality controls of the milk,[11,214,215] environment, devices, and the involved staff.[180,212,216–220]

Following the correct procedures along the entire supply chain (from the extraction to the preparation of

the feedings), the routine cleaning of the environment, and the decontamination of the devices and materials in use, has a great impact not only on the microbiological safety but also on the organoleptic characteristics of the milk and on the duration of storage.

3.1. Bacteriologic screening and quality control

Bacteriological and quality controls of the donated milk are extremely important for the safety of the recipients. Even if a general agreement on the criteria of acceptability of the milk has not yet been reached, the most commonly accepted microbiological values are reported in Table 10.

Evidence for recommendations 3.1.1–3.1.5

3.1.1. These checks help reduce the physical, chemical, microbiological risks of the dietary products according to the HACCP principles (see the earlier sections on Organization and Autocontrol and hazard analysis and critical control points). They are considered CCPs only if applied in a systematic way to all the milk samples, otherwise they are defined as GMPs.

3.1.2. There is no strong evidence in favor of systematic bacteriological screening before and after pasteurization. Carrying out bacteriological tests can result in unfairly discarding a substantial quantity of milk, because, bacteriological screening itself, if not performed in appropriate conditions, can lead to contamination.[11,87,221]

Currently, there is no consensus on the screening schedule or on the microbiological criteria to be used to define the acceptability of the milk before and after pasteurization.[11,12,14,82–85,222]

Evidence for pre-pasteurization testing. The microbiological tests are aimed to identify the milk contaminated by a higher number of aerobic microorganisms (pathogenic or nonpathogenic) than Holder pasteurization can eliminate with a reasonable safety margin; the pasteurization process itself has the potential to alter the quality of the milk. Milk contaminated with

Table 10 **Recommendations for bacteriological screening and quality control**

Recommendation	Quality of evidence	Strength of recommendation
3.1.1. The first quality analysis should be done at the first moment the staff takes charge of the milk. It is essential to evaluate if the container is appropriate and intact, if it is properly labeled, and if the milk is still frozen.	++++	↑↑
3.1.2. The recommended schedule for bacteriologic testing is:		
a. Pre-pasteurization testing: It is recommended to test some of the milk samples of 1 mother at the beginning of the donation. If the first test results do not fulfill the standards defined, it is appropriate to review the hygienic norms together with the donor, and then repeat the bacteriologic test. Further noncompliance entails exclusion from donation. It is recommended to repeat the bacteriological analysis during the donation period with a frequency appropriate to the case.	+++	↑↑
b. Post-pasteurization testing: It is recommended to test the pasteurized milk samples in a random and regular way (eg, once a month, once in every 10 pasteurization cycles). In case of the implementation of new procedures or devices, or of events raising concerns about reliability of the staff, procedures, devices, or any other part of the process, it is recommended to perform additional tests (pre- and post-pasteurization). When bacteriologic screening is performed on a sample from a batch, the entire batch should be quarantined; it can be used only when the culture results are known to be negative. Otherwise, the batch is discarded or used for research purposes.	+++	↑↑
3.1.3. Criteria to define acceptability of the milk when bacteriologic analysis is performed are the following:	+++	↑↑
a. Before pasteurization: The milk is discarded if it contains: • Total viable bacteria $>10^5$ CFU/mL, or • Enterobacteriaceae $>10^4$ CFU/mL, or • *Staphylococcus aureus* $>10^4$ CFU/mL		
b. After pasteurization: The milk must be discarded if it contains: • Aerobic bacteria $\geq$10 CFU/mL		
3.1.4. It is essential to check periodically, with quality control tests (including microbiological samples taken from the environment, devices, the involved staff members), all the procedures of the HMB according to the HACCP principles.	+++	↑↑
3.1.5. Quality control aiming to reveal possible manipulations or fraud due to the addition of cow's milk can be performed in a casual way, in case of suspicion.	++	↑

Abbreviation: HACCP, Hazard Analysis Critical Control Points; HMB, human milk bank.

Staphylococcus aureus and Enterobacteriaceae, including *Escherichia coli* and/or *Pseudomonas aeruginosa* (pathogens that can produce enterotoxins and thermostable enzymes), should be discarded if the level of contamination causes a risk for toxi-infection (the theoretical risk for a bacterial load is $>10^4$ CFU/mL for these organisms). However, these bacteria are destroyed by pasteurization, and harmful clinical effects on neonates have not been reported. A strict control of the procedures described in this section will ensure the safety of the milk in terms of these theoretical risks.[223–225]

Expression and storage of the milk at home are the phases where the milk is exposed to the major risk for bacterial contamination. HM expressed at home is more contaminated than milk expressed in the hospital.[216–218,226] At the beginning of the donation, milk expression at home can be safe,[227] but with time, the mothers can become less rigorous in following hygienic norms.[228] It also is important to note that most of the inappropriate samples (ie, those heavily contaminated and/or with pathogenic bacteria) come from a limited number of donors.[229,230] The recommendation of bacterial screening at the initiation of the donation (1, or preferably 3 in successive days) is in line with recommendations of other guidelines and authors.[84,222,225,229]

The current approach is focused on the quality control of the entire process of milk handling, from expression until the reception by HMB staff, and regular controls are sufficient. Correctly instructed donors usually supply a product fulfilling microbiological safety requirements. A repeated nonnormal test result from a donor can lead to exclusion from the donation.[84,225]

When bacteriologic screening is performed, the expressed and not-yet-tested milk samples should be quarantined (frozen) in the meantime and discarded if the bacterial counts of the analyzed samples are unacceptable.

Formation of a pool of milk prior to the pasteurization, by mixing the milk samples either from the same donor (sub-batches) or from a limited number of donors,[3,4] offers an advantage of balancing and reducing the concentrations of toxic and infectious agents that possibly can be present in the milk,[11] making the heat treatment more effective. However, the disadvantage arises in the case of a contamination, where it can be more difficult to identify the origin of contamination.

The recommended approach has a limit of not providing information for all the samples or batches of milk undergoing pasteurization, but it allows a sufficiently adequate quality control of the process of the milk handling until the moment that HMB staff takes charge of it. The recommended approach also simplifies the procedures and reduces elimination of an entire batch of milk, which is very precious, and which is usually being discarded unfairly otherwise.[11,84,87,221]

Evidence for post-pasteurization testing. To check that potentially risky bacteria survived pasteurization (a rare event) or the contamination immediately after the pasteurization (a rare event), all batches would require testing. Using the proper culture medium, low loads of spore-forming bacteria, such as *Bacillus cereus*, can be rarely detected in the pasteurized milk.[211,231] Pasteurization destroys the vegetative forms, but not the spores, which can germinate under heat effect. *B. cereus* is a ubiquitous germ that could be pathogenic in immune-compromised infants and produces a thermostable toxin. However, the theoretical risk for the recipient is very low.[219,232] In fact, toxi-infections due to the use of contaminated DHM have never been reported to our knowledge. We believe that systematic testing is not necessary. The contamination and growth of *B. cereus* in the milk, like the other microorganisms, can be prevented by controlling the environmental hygiene of the HMB (note the importance of the microbial testing of environment), and hygiene at the donor's home, by checking the devices, and by proper handling of the milk after pasteurization (ie, rapid chilling, careful manipulation, and storage).[11]

Thus, it is recommended to perform post-pasteurization microbiological testing in a predefined, regular way, and for randomly selected batches, not of the single samples, as a part of quality control of the process of milk handling. There is no strong evidence to recommend testing all the batches of milk after pasteurization.[11,84]

3.1.3. There is no consensus on the microbiological criteria to be used to define the acceptability of the milk before and after pasteurization.[11,82–85,222] It should be remembered that the threshold criteria for bacterial contamination above which the milk should be discarded are based on the knowledge of the experts and on the criteria used for the food industry.

3.1.4. A strict application of the HACCP principles, from the collection to the distribution of the donated milk (including quality controls, microbiological tests on the devices, environment, and the involved staff) is fundamental to ensure safety for the recipient and to minimize discarding the milk.[212,216–220]

UK guidelines[11] recommend that post-pasteurization microbiological tests be performed regularly (checking total bacterial load) either once a month or once in every 10 cycles, which comes first.

When bacteriologic screening is performed in a sample rom a batch, the entire batch should be quarantined while waiting for the results. The milk samples

can be stored in the refrigerator for a maximum of 48 hours or can be frozen.[83]

3.1.5. Detection of cow's milk protein is done via validated immunological tests.[83]

3.2. Pasteurization methods

Pasteurization is a process of thermal sanitation, which, by combining in an appropriate way temperature and treatment duration, aims to reduce health risks deriving from the presence of heat-sensitive microorganisms, including vegetative bacteria, viruses, fungi, and yeasts in the milk (or other dietary products).

If performed correctly, pasteurization inactivates pathogen bacteria, reduces the total microbial load, and inactivates several enzymes (eg, proteases, lipases), thus stabilizing and making the milk more appropriate for storage. Compared with the sterilization, which eliminates all microorganisms and spores but alters drastically the biological characteristics of the food, pasteurization is less harsh to precious bioactive and nutritional components of the milk, causing a moderate and acceptable reduction.

Efficacy of the process depends on various factors. A correct combination of the temperature and time is certainly essential to reduce the bacterial count to a safe value. Additionally, the initial concentration of the microorganisms and their resistance to the heat influence the outcomes of the process. For each set temperature and for each specific population, the initial number of microorganisms decreases by a constant percentage per time unit (eg, seconds, minutes).

The greater the number of microorganisms to inactivate and the greater resistance they have to heat, the more time will be necessary to render them harmless. In some conditions, 1 cycle of pasteurization may not be sufficient to eliminate completely a population of bacteria.[213,234,233] Hence, it is necessary to underline once more the importance of adherence to the correct procedures for milk handling along the entire supply chain to reduce the growth of microorganisms.

The pasteurization method using a low temperature for a long time, in other words Holder pasteurization (62.5°C for 30 minutes), is the most widely studied and historically the most widely used method for the pasteurization of various foods. It also is recommended for the treatment of DHM.[11,12,14,82,83,222] Holder pasteurization inactivates most of the pathogenic microorganisms with a reasonable margin of safety (including the bacteria resistant to heat, such as *M. tuberculosis* and *Coxiella burnetii*). The Holder method offers a good compromise between the microbiological safety and nutritional and biological quality of the milk.

The alternative techniques that are currently being studied, are high temperature for a short time, high-pressure processing, and ultraviolet-C irradiation. They can be applied both to solid and liquid foods. High temperature for a short time pasteurization (ie, +72°C for 5–15 s), provides the best compromise between the microbiological safety and nutritional and biological quality of the milk. However, this method requires the use of a device currently available only at the industrial level.[235] There is still a shortage of the research comparing the other innovative techniques using thermic and nonthermic processes. Moreover, the data related to the microbiological safety of the new technologies are still scarce, particularly for ultraviolet-C irradiation and for thermic ultrasound.[236,237]

All the milk arriving at the HMB must be pasteurized. The ideal pasteurization cycle should consist of a phase of rapid heating followed by a phase of constant maintenance of the temperature and a final phase of rapid cooling. The specific parameters indicating the correct functioning of the pasteurizers should be checked regularly, at least once a year[155] (GMP), to optimize the results of the pasteurization process[238] (CCP).

Pasteurization of the milk from a single donor makes traceability of the milk easier. Making a pool from the milk samples of the same donor (a sub-batch) or of a limited number of donors (n = 3–4; a batch) prior to the pasteurization offers advantages from a nutritional point of view. Pooling allows also dilution of possible toxic and infectious factors[11] and makes the pasteurization more effective and safer. Ensuring the traceability of the process is essential, and pooling must not be done with pasteurized milk.[85] The characteristics of the pasteurization process are described in Table 11.

Evidence for recommendations 3.2.1.–3.2.5

3.2.1. Milk containers with different volumes of milk reach the initial pasteurization temperature at different times.[11] The freezing process after pasteurization (carried out if the milk is not used within 48 h) increases the liquid volume in the container; for this reason, it is recommended to fill the bottles with milk up to a maximum of four-fifths (80%) of their capacity.

3.2.2. Pasteurization at 62.5°C for 30 minutes (the Holder method) destroys the pathogenic bacteria in the milk, including *M. tuberculosis*, as well as fungi, many viruses (eg, HIV-1, HTVL I/II, CMV, herpes simplex, rubella, SARS-CoV, SARS-CoV-2, Middle East respiratory syndrome–related coronavirus).[11,80,139,142,144,145,155,239,240]

The Holder method reduces the concentration of some immunologic and anti-infective factors: IgAs, IgG, IgM, lysozyme, lactoferrin, and complement.[198,205,225,241,242] Some macronutrients (eg, protein,

Table 11 **Management of the pasteurization process**

Recommendation	Quality of evidence	Strength of recommendation
3.2.1. Heat treatment must be carried out on fresh, not frozen, milk in sterile and tightly closed milk containers. The bottles should contain similar volumes of milk, up to a volume not more than four-fifths (80%) of the capacity of the bottle. Before the pasteurization process, organoleptic characteristics of the milk should be evaluated. In case of an improper odor or appearance, or if foreign bodies are present in the milk, the sample should be discarded.	+++	↑↑
3.2.2. For human milk banks, pasteurization at 62.5°C for 30 min (Holder method) is recommended. Currently, pasteurization at a lower temperature is not acceptable.	+++	↑↑
3.2.3. It is necessary to measure, check, register, and keep the data regarding the heat-treatment cycle. A control bottle, with the same amount of milk as in the other bottles of the batch, located in the center of the water bath of the pasteurizer, should contain an immersion thermometer to register milk temperature during the pasteurization process (∼25% of the milk volume must be below the measuring point of the temperature probe). Initiation of the pasteurization process is calculated from the time when the milk inside the control bottle reaches the desired temperature of 62.5°C. The heat treatment must continue for 30 min at this temperature.	+++	↑↑
3.2.4. The final phase of the pasteurization cycle must provide a rapid cooling of the milk from 62.5°C to 25°C in <10 min. The milk should reach, as quickly as possible, a temperature of 10°C, preferably of 2–4 °C.	+++	↑↑
3.2.5. The bottle caps must remain above water level to prevent possible contamination in case the closure is not hermetic.	++	↑↑
3.2.6. At the end of the pasteurization cycle, the baskets containing the milk should be removed immediately from the pasteurizer.	++	↑

lipids) and energy can be reduced as well (but can be replaced by the fortification).[243] However, with Holder pasteurization, some of the key nutritional factors (namely, lactose, long-chain polyunsaturated fatty acids, fatty acids, gangliosides), and some other bioactive factors (namely, oligosaccharide, amylases, epidermal growth factor) and vitamins A, D, and E remain unchanged.[244–246]

3.2.3. The system of HACCP foresees the measurement and registration of the heat-treatment processes applied to the food. Controls are important to ensure the safety and high quality of DHM: the pasteurizer should follow precisely the program, time, and temperature set for the process.[219,238]

3.2.4. The rapid cooling phase provokes a thermic shock to the bacterial content without altering the immunological components.[11,247,248] This rapid cooling velocity results in avoiding bacterial growth.[11,236]

3.2.5. In the phase of cooling, *Pseudomonas aeruginosa* contamination has been reported.[249]

3.3. Storage of milk at the human milk bank

The milk arriving at the HMB should be kept in dedicated refrigerators and freezers, equipped, if possible, with remote temperature control or, alternatively, a thermoregistration system, and should have temperature-sensitive acoustic and visual alarms that can be observed in real time by the staff. The refrigerators should maintain a temperature of +2 to +4°C, and freezers a temperature of not warmer than –20°C. Exposure of the milk to light should be avoided except for the procedures necessary for the preparation of meals. Table 12 describes how to handle and store the milk in the HMB.

Evidence for recommendations 3.3.1.–3.3.4

3.3.1. Labeling enables identification of the HMB, the single donor, and the time between milk collection and pasteurization.[12,82,250] The traceability system with a barcode minimizes the risk of an error in the use of maternal or donor milk.[251] In the case of mothers having the same or similar names, it is recommended to use warning labels.

3.3.2. The nonpasteurized milk can be subject to organoleptic alterations due to the presence of bacteria that ferment lactose, resulting in the production of lactic acid, and metabolize proteins. The proteases cause reduction in the proteins, whereas lipases, with persistent activity during refrigeration and freezing, cause lipid hydrolysis, leading to an increase in free fatty acids and osmolarity, and reduction in pH. Pasteurization destroys bacteria and inactivates lipases and proteases, ensuring organoleptic stability of the milk.[206,252,253] For this reason, it is recommended to carry out the pasteurization as soon as possible both for the milk expressed at home or at the HMB. For the milk collected at the

Table 12 **Handling and storage of the milk at the human milk bank**

Recommendation	Quality of evidence	Strength of recommendation
3.3.1. At the moment the HMB staff assumes the responsibility of the milk, it should be verified if the milk expressed at home and transported to the HMB is still frozen, if the milk containers are appropriate and intact, and if there are labels identifying the HMB, the donor, and the collection date.	+++	↑↑
3.3.2.		
a. The milk expressed at home, following acceptance at the HMB, should be placed immediately in the refrigerator while waiting for pasteurization, which should be carried out as soon as possible within 24 h. If a longer waiting time is foreseen for pasteurization, the milk should be frozen as soon as possible.	++++	↑↑
b. Fresh expressed milk collected at the HMB should be transferred immediately to the refrigerator and pasteurized as soon as possible, preferably within 24 h, and not later than 72 h. If a waiting time longer than 72 h is foreseen for pasteurization, the milk should be frozen as soon as possible.	+++	↑↑
3.3.3. Both fresh and pasteurized milk stored in the freezer at a temperature ≤20°C should be used within maximum 6 mo after the date of expression. When DHM is used for feeding preterm neonates, the recommended period for storing frozen milk is a maximum of 3 mo after the date of expression.	+++	↑↑
3.3.4. Cooled or thawed pasteurized milk can be stored in the refrigerator at +2 to +4°C, and should be used preferably within 24 h, and within 48 h maximum.	++	↑↑

Abbreviations: DHM, donor human milk; HMB, human milk bank.

HMB, however, it is acceptable to store it in the refrigerator at +2 to +4°C for a maximum of 72 hours, because, in this context, appropriate hygienic and safety conditions can be ensured.

3.3.3. Freezing at −70°C is considered the gold standard for long-term milk storage. However, this method requires very expensive freezers and they are not available in most of the HMBs.[198,254] There is agreement in the literature on a freezing temperature between −20°C and −25°C for milk storage (the temperature reached by most of the freezers used at home and at HMBs). However, there is no agreement on the maximum storage time of the milk at this temperature, with suggested storage times varying from 1 to 12 months.[198,255,256]

It has been demonstrated that freezing at −25°C for 3 months causes a minimum loss of the biologic activity of HM.[257] Freezing at −20°C for 3 months does not affect secretory IgA, IgG, C3, lysozyme,[142] or lactoferrin, or nutritional factors such as amino acids, lipids, α-tocopherol, γ-tocopherol, and retinol.[258] It slightly reduces levels of vitamins B_6 and C, IgM, IgG, bacteriostatic activity, C3, lipases, and number and function of the cells.[198,254,259]

A longer storage period at −20°C causes an increase in free fatty acid concentration, due to the destruction of fat-globule membranes.[204,205] Although these free fatty acids have a cytolytic effect on pathogenic organisms,[198,203] they can cause the milk to become rancid.[260] This temperature also causes a significant decrease in fat, energy, vitamin C content, and alterations in the volumes of fat-globule membranes.[261,262]

3.3.4. Pasteurization reduces the bacteriostatic activity of the milk, favoring bacterial growth, if the bacteria have not been completely eliminated or when a contamination occurs after pasteurization. Some studies suggest that pasteurized milk, after being cooled or thawed, can be stored in the refrigerator for 96 hours or longer. However, prudentially, it is recommended to use this milk within a maximum of 48 hours.[206,253,263,264]

3.4. Thawing methods

Both milk expressed and frozen at home and milk pasteurized and frozen at the HMB undergo a thawing process. Because pasteurization and freezing reduce the bacteriostatic and bactericidal activities of milk, pasteurized thawed milk should be handled very cautiously according to the principles of HACCP. Thawing methods are listed in Table 13.

Evidence for recommendations 3.4.1.–3.4.5

3.4.1. At a temperature of +2 to +4°C, the bacterial count of fresh milk does not increase significantly in 24 hours.[200,265] Slow thawing is associated with a minor reduction of the lipids.[265,266]

3.4.2. In the case of thawing with running water or in a water bath, there is a risk of milk contamination if the cap of the container is not thoroughly sealed.[12,266–269]

3.4.3. Germs present in HM multiply very rapidly at room temperature.[137,138,142] The bactericidal property of the milk is decreased with freezing.[270] Prior to

Table 13 **Thawing methods**

Recommendation	Quality of evidence	Strength of recommendation
3.4.1. Milk thawing can be done: • Slowly, in the refrigerator at +2 to +4°C for a maximum period of 24 h • Rapidly, in a water bath at a temperature not exceeding 37°C, or under running lukewarm water	++	↑
3.4.2. Rapid thawing of the milk should be carried out with particular attention to avoid the contact between the cap of the container and the water.	++	↑
3.4.3. The milk expressed and frozen at home, after being thawed at the HMB, should stay at room temperature for the minimum possible time, or it can be stored in the refrigerator for a maximum of 24 h before pasteurization. The pasteurized frozen milk at the HMB, after thawing, should be used for feeding as soon as possible (within 4 h if kept at room temperature), or it can be kept in the refrigerator at +2 to +4°C for a maximum of 48 h.	++	↑
3.4.4. Fresh frozen milk should not be refrozen after being thawed.	++	↑
3.4.5. It is contraindicated to thaw the milk in a microwave oven.	++	↑

Abbreviation: HMB, human milk bank.

pasteurization, the milk should be stored in the refrigerator for a maximum of 24 h.[271–273]

3.4.4. Repeated freezing and defreezing cycles increase hydrolysis of triglycerides.[274,275]

3.4.5. Heating milk in a microwave oven significantly reduces vitamin C, total IgA, *Escherichia coli*, and specific IgA levels, and the activity of lysozyme in the milk.[276,277] In addition, if the milk is used within a short time, there is a risk of causing burns to the infant, because the temperature of the milk is not homogenous and can be higher than that estimated.[265,276]

3.5. Distribution of the milk

Donated milk is prioritized for VLBW neonates. It can be used also for the nutrition of other sick, vulnerable infants and clinical situations (see the earlier subsection Other Clinical Uses of Donor Human Milk).

DHM is distributed with a medical prescription to hospital neonatal units and pediatric departments or to other HMBs when there is a requirement. In exceptional situations, it can be given also to the patients at home. The HMB staff register the request (on paper or electronically), including identity of the recipient; date of distribution; the number and the volume of the distributed bottles; and the type and, if possible, the number of recipients (eg, VLBW, preterm infants weighing >1500 g, neonates with medical problems, healthy neonates awaiting MOM production).

The aspect of the product, the integrity of the container, the label (traceability), and the expiration time should be checked from the person who receives the milk and the person who is in charge of transportation. All these items should be registered in the document accompanying the milk during transportation.

Milk transportation should be performed strictly following cold-chain rules.

CONCLUSIONS

The present recommendations have the main goal of guaranteeing a quality standard for the operation and management of an HMB in the different phases of the production chain: from donor selection, to the collection, processing, storage, and distribution of DHM. The recommendations are based on the scientific evidence existing at the moment for the different aspects of HM donation and HMB management.

It is extremely important to underline that HMBs are not only centers for collection, processing, storage, and distribution of DHM. They represent a unique opportunity to promote, protect, and support breastfeeding. The World Health Organization and UNICEF issued a joint statement in 2003 mentioning that the opening of an HMB is part of the initiatives taken, at international level, to promote and support breastfeeding:

> *The vast majority of mothers can and should breastfeed, just as the vast majority of infants can and should be breastfed. Only under exceptional circumstances can a mother's milk be considered unsuitable for her infant. For those few health situations where infants cannot, or should not, be breastfed, the choice of the best alternative – expressed breast milk from an infant's own mother, breast milk from a healthy wet-nurse or a human-milk bank – depends on individual circumstances.*[2]

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interpretation of the collected data. L.G., E.B., and G.S. defined and proposed GRADE classification for the levels of evidence and formulation of the recommendations. S.A. wrote the draft version of the paper. G.E.M. revised the article critically for the intellectual content.

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