

Guidelines for the use of human milk and milk handling in Sweden

2016-04-01 Milknet, version 3.0

Translations to English: Version 2.0/2011 was translated 2013 by Margot Lundqvist, and version 3.0/2016 was translated 2017 by the authorial group (responsible S Polberger).

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Table of contents	Page
Term definitions	2
Background	2
Donor milk	3
Target groups for use of donor milk	3
Prerequisites	3
Recruitment of donors	3
Health declaration	4
Serological screening	4
Bacteriological testing	4
Pooling of milk	5
Pasteurisation (heat treatment)	5
Unpasteurised donor milk	6
Labeling/coding	6
Transportation between hospitals	6
Financial compensation	6
Milk kitchen/Human milk bank procedures	6
Nutritional assessment of donor milk	7
Mother's own expressed milk	7
Nutritional assessment of mother's own milk	8
Practical handling of breast milk	9
Freezing	9
Storage	9
Defrosting	10
Warming	10
Pumping/nursing room	10
Hygiene rules	11
Fortification of breast milk	11
References	13
Appendix 1. List of human milk banks in Sweden, 2014	18
Appendix 2. Health declaration of breast milk donor (example)	20
Appendix 3. Pasteurisation of donor milk (example)	22

Term definitions

- Donor milk = breast milk from a woman who is not the biological mother of the recipient of the milk.
- Mother's own milk = breast milk from the infant's biological mother.
- Preterm milk = milk from a woman who has given birth to a preterm infant (born before 37 weeks of gestation). The composition of certain nutrients (e.g. higher protein content) can differ from that of the milk of mothers of full-term infants for varying periods of weeks or even months.
- Pooled milk = donated milk from several individual women, pooled before use.
- Human milk bank = facility that handles and supplies breast milk to medical services.
- Lactation = breast milk production. The breast milk is received by the infant directly via nursing or after having first been expressed from the breast and collected in an appropriate container.
- Pasteurisation = heat treatment.

Background

Breast milk is the first choice for feeding newborn infants, and that choice has a long tradition in Sweden and in Scandinavia, whereas in other countries infant formula has been widely used. In the 1900s (starting in 1909 in Vienna), human milk banks were established in many countries to handle the milk of mothers other than an infant's own, primarily in neonatal care (1). Many of these banks were phased out during the 1980s due to the fear of transmission of HIV. However, many have later been re-established, and the number of milk banks is now increasing (2-4). It has also been observed that there is an increased use of mother's own milk if there is a human milk bank linked to the neonatal unit (5).

The knowledge of the advantages of human milk compared to infant formula, for both term and preterm infants, is now better recognized. This is attributable to both mother's own and donor milk (6-10).

Access to donor milk is currently limited. Most of the donated milk in Sweden today is used for the nutrition of preterm infants (11).

In 2001 a network called the Swedish Milknet was formed of representatives of neonatal care (dietitians, assistant nurses, neonatal nurses and neonatologists). Regular meetings have been held in various hospitals throughout Sweden. Its purpose is to maintain and improve access to donated milk, and to exchange experiences in breast milk handling and breast milk feeding of newborn infants. A primary tangible ambition was to create these uniform guidelines for Sweden for practical breast milk handling in neonatal care; the guidelines are based on earlier recommendations (12). National guidelines exist e.g. from the United Kingdom, the United States, Norway and Australia (13-16). An authorial group composed of representatives of the country's various profession branches in neonatal care has been formed, and these recommendations are the result of the group's efforts. The first version was published in March 2008 with a first revision in 2011 (17), and this is the English translation of the second revision published in 2016 (version 3.0).

In Europe there is a network of representatives from human milk banks, and in October 2010, after several years of meetings, it established the European Milk Bank Association (EMBA). Its headquarters is in Milan, and its website is www.europeanmilkbanking.com. The first board was made up of 11 members from 9 European countries. Sweden was until 2016 represented by Staffan Polberger. The board now consists of 11 persons from 8 countries. The only Nordic representative is Anne Grövslie from Oslo, Norway, who is also the secretary of EMBA and a member of the Swedish Milknet.

Donor milk

Target groups for the use of donor milk

Donated milk is used primarily for preterm infants during their first days, until mother's own milk production is established. It is also used for infants in later stages of prematurity, if the mother cannot produce sufficient amounts of milk, for any reason cannot produce milk at all, or the milk cannot be used. In certain cases other groups of infants can be given donated milk. In Sweden almost all handling of donor milk is performed in neonatal care units.

Appendix 1 is a telephone and e-mail list of the 28 national human milk banks, with information about their activities during 2014.

Prerequisites

- There shall be a medically responsible physician for the local milk bank.
- All use of donor milk shall be prescribed in writing by a physician.
- Use of donor milk cannot be started without the consent of at least one of the parents of the recipient infant. The approval shall be documented in writing in the medical records.
- Use of donor milk shall be registered daily in the child's medical record, including the donor code, so that it is possible to trace the donor in case of infection.
- The staff taking care of the milk handling should if possible have no other responsibilities in the neonatal unit.

Recruitment of donors

All healthy women who produce breast milk can be presumptive donors to a human milk bank, but there are a number of **exclusion criteria**:

1. Tobacco smoking or use of snuff, regardless of quantity.
2. Use of any kind of illegal drug.
3. Use of any kind of medication, including certain naturopathic preparations (up to individual judgment).

However, milk donation can be accepted despite treatment with certain medications:

- Substitution treatment with various hormones, e.g. thyroid hormones and insulin.
- Topical asthma treatment with inhaler.
- Topical treatment of skin, nose, and eyes.
- Contraceptives in progestogens form.
- Occasional use of analgesics.

During systemic antibiotic treatment (perorally or injection), the collections for the milk bank shall be temporarily suspended until 24 hours after completed treatment.

All medication consumption, including that of naturopathic preparations, vitamins, homeopathic preparations, and herbal preparations shall be reported to the milk bank. Temporary suspension of milk donation can be necessary.

Donor use of alcohol: Occasional limited consumption can be accepted.

Donor use of coffee and caffeine-containing beverages is acceptable in moderate amounts (up to e.g. 3 cups per day).

The following groups of women are unsuitable as milk donors:

1. Those who have earlier been or are presently illegal drug abusers.
2. Those who have had any transfusion of blood cells or other blood products in the past 6 months.
3. Those who have had tissue or organ transplantations in the past 12 months.
4. Those who have had body-piercing or tattooing in the past 6 months (except for earrings).

5. Those who have had a sex partner during the past 12 months who is suspected of being a carrier of HIV, HTLV, or hepatitis or who has used injection needles for illegal drugs, or is a known hemophiliac. Those who have chronic infections themselves such as HIV, HTLV, hepatitis B or C, malaria, or active tuberculosis.
6. Those who have any cancer disease (with the exception of localized skin cancer that is not melanoma or cervical cancer in situ).

Breast milk donation should be temporarily suspended in connection with:

1. All acute infections, including mastitis (mammary gland inflammation) and diffuse infection around the nipple.
2. Reactivated herpes or zoster infection (shingles).

Acupuncture and electrolysis done with sterile disposable needles are acceptable.

A milk donor's diet should be balanced. Women who have non-conforming diets that eliminate several important staple foods shall not be accepted as breast milk donors before the dietitian has made an assessment. A donor should have a daily breast milk production of at least 500 mL.

Health declaration

Before a woman can be accepted as a donor, she shall have completed a health declaration that is to be approved by the responsible physician of the local milk bank. A sample health declaration is attached as Appendix 2.

Serological screening/other examinations

All presumptive donors are screened with blood tests for HIV-1, HIV-2, HTLV-I, HTLV-II, hepatitis B, and hepatitis C. Breast milk from donors shall not be used until negative results of these tests are available. If the results of any of these analyses are positive, local routines shall exist to take care of the woman rapidly to avoid unnecessary worry. Syphilis serology has been done at the maternity clinic and need not be repeated.

If the donor is to continue providing milk for longer than 3 months from the infant's birth, this serological screening shall be repeated, and the milk shall not be used until the new negative results are available (16).

Bacteriological testing

Before a woman can be approved as a donor, the milk must undergo a bacterial culture test as a hygiene control. Alternatively, the milk can be examined at the pasteurisation procedure as described below.

The sampling is done most easily as a scrape test, using a sterile medical spatula or similar device to scrape a sample from the frozen milk – or can be taken from the fresh milk, and transferred to a bacterial testing tube. The milk does not need to be defrosted in order to obtain a representative milk sample for bacterial analysis.

To be accepted as a milk donor the following bacteriological requirements on the milk sample must be met:

- No potentially pathogenic bacteria such as beta-hemolytic streptococci group A, C or G, streptococci group B, Listeria, or Salmonella
- $< 10^7$ CFU/L Staphylococcus aureus
- $< 10^7$ CFU/L Gram-negative bacteria (Enterobacteriaceae)
- $< 10^7$ CFU/L Pseudomonas aeruginosa or other Pseudomonas species
- $< 10^7$ CFU/L Stenotrophomonas maltophilia
- $< 10^7$ CFU/L Acinetobacter species

- There is no upper limit for total aerobic count, including e.g. coagulase-negative staphylococci or alphahemolytic streptococci.

If the requirements are not met, a new sample is taken for culture after the hygienic procedures have been discussed with the donor, which usually leads to approval. If not, the donor is disapproved. During continued milk donation, bacterial testing shall be performed once a month as a donor hygiene check.

As an alternative to the early hygiene control you can choose to analyse the bacteriological quality of the milk immediately **before** pasteurisation. Take a well-mixed sample from the entire milk volume in a pasteurisation vessel before the milk is distributed in different bottles. The advantage with this technique is that you obtain information from the entire milk volume and not only from one random sample; however, the disadvantage is that if the milk is disapproved, you have to discard the entire milk volume. The cut-off points are identical to the ones mentioned above.

For further control **after** the pasteurisation procedure you may perform systematic random sample controls (process control according to standard ISO 2859-1), but there are no strong reasons for such a recommendation. A study showed that 93% of the milk cultures were sterile after Holder pasteurisation (18). If you can find bacteria the total aerobic count must not exceed 10^4 CFU/L (13). Naturally, no pathogenic bacteria are accepted.

Pooling of milk

Pooling of milk can not be recommended. Pooling involves blending the milk of several donors to achieve a more homogeneous milk composition, when no routines for human milk nutrient analyses existed. Nowadays, when such analyses can be done and followed by individualized fortification, there is no reason to pool human milk. For Swedish infants there is data that pooled donor milk will result in a lower content of macronutrients compared to milk from individual donors (19).

In almost all neonatal units in Sweden donor breast milk from individual mothers is used in order to limit the individual infant's exposure to a number of donors. Using this system means that there can be large variations in the donated milk's nutritional values. When nutritional analyses are done, the most appropriate milk can be chosen, for example the most protein-rich donor milk for a newborn, very immature infant. With pooling the possibility of choosing an extra protein-rich milk is lost, and the possibility for tracing infections is made more difficult.

Pasteurisation (heat treatment)

Almost all donated milk is pasteurised (heat-treated) in Sweden prior to use. The most frequently used method is rapid heating to 62.5°C for 30 minutes followed by rapid cooling to room temperature – the Holder pasteurisation method, using specific equipment.

Holder pasteurisation (Appendix 3) kills all viruses (with some uncertainty about hepatitis B), as well as almost all bacteria (18). At the same time it reduces the activity of the antibacterial protective factors in milk, such as sIgA (secretory IgA), lysozyme and especially lactoferrin (20). Macrophage activity ceases, as does lipase activity in the milk, which leads to decreased absorption of fat (21).

Other methods of pasteurisation are being discussed, but are not used in Sweden. Examples of these are heating at 57°C for 30 min (22), 62-75°C for 5-15 sec (23-25), or 56°C for 15 min (26).

Microwave ovens are not suitable for pasteurising, for defrosting, or for warming milk, as the temperature distribution can be uneven or the temperature can become too high, as well as possibly presenting an increased risk of bacterial growth (15, 27-29).

If breast milk in a neonatal unit is subjected to bacterial contamination, it must be remembered that the risk of bacterial growth is greater in heat-treated milk than in milk that has not been heat-treated. This is because some of the milk's bacteriostatic properties have been damaged by the pasteurisation process (20).

Unpasteurised donor milk

If an individual infant, for example a very immature infant, should receive unpasteurised donor milk for medical reasons, this option is still possible (20). If there are no such reasons, the donated milk shall be pasteurised. In contrast, unpasteurised donor milk is widely used in some countries such as Norway and parts of Germany (30).

Donor milk that is used unpasteurised shall not be pooled. Use of unpasteurised donor milk requires following very strict hygiene rules, and demands awareness that a risk of cytomegalovirus (CMV) contamination will necessitate serological testing of the donor. See discussion under "Mother's own expressed milk", below.

Labeling/coding

All donor milk shall be labeled in such a way that it is possible to trace it back to the donor in case of contagion. It shall also be possible to track down which infants have received which donor milk. When the milk is pasteurised a code shall be assigned to the individual donor. For example, a bar code system can be used. Alternatively, the milk can be coded using a marking such as "09-15C", where the "09" stands for the year the donor was recruited, the "15" stands for the donor, and "C" shows that this is the third batch of milk that is pasteurised from this individual donor (this is the system used in Lund). In the milk bank's documentation there should be notations of the time period in which the milk was collected. Considering the periodical shortages and the increased exchange of donor milk between hospitals, it would be useful to have uniform labeling throughout the country.

There is an ongoing development towards standardized routines for labeling of milk in a comparable way to that being used in the blood banks in Sweden. We have to consider if we should strive for such a change (for increased traceability in a case of contagion).

Transportation between hospitals

Donor milk can be transported to other hospitals if it remains frozen for the whole transfer. The transfer time shall be registered.

Financial compensation

Rental for the breast pump is always paid by the healthcare system if the mother is expressing milk for medical reasons (such as a sick infant in the neonatal unit), or is a donor to the milk bank. Delivery and return of the pump as well as access to servicing of the pump shall be available close to the neonatal care unit and milk kitchen at the hospital.

In Sweden a varying financial compensation per liter of 100-250 SEK (about 12-30 USD) is paid to the donor. This compensation is tax-exempt (Income Tax Act 1999;8:29). Uniform compensation rules for the entire country should be strived for, and is recommended to be 250 SEK per liter. See Appendix 1 for the situation in Sweden in 2014.

Milk kitchen/Human milk bank procedures

Staff who handle donor milk, for example during pasteurisation, shall not be engaged in the care of sick infants at the same time. It is also desirable that staff who handle breast milk for measurement, fortification, etc. are not caregivers of sick infants at the same time.

The following information about breast milk donors shall be kept available and shall be archived for 30 years:

- Declaration of health.
- Results of serological screening and other testing.
- Results of bacteriological tests of the milk.
- Data on coding of the donor, collection dates of milk, and volumes.
- Finances, compensations paid.

Nutritional assessment of donor milk

Donor milk is usually analysed for protein, fat, and carbohydrate contents, which also gives a calculated energy value. A sample is taken from carefully stirred milk. When the analysis is done with infrared technology, the sample is always taken before pasteurisation as there is otherwise a risk of incorrect results because of the heat treatment. The results of the analysis are documented in such a way that they are available to care staff around the clock.

Mother's own expressed milk

A mother can almost always give her infant her own breast milk, and in Sweden only mothers who have active tuberculosis or are HIV-positive are advised not to nurse their infants. Exclusive breastfeeding works only for reasonably healthy full-term infants, and the sick or preterm infant (<34-35 weeks gestational age) is at least partly dependent on provision of breast milk via a nasogastric tube.

In order to supply the infant with mother's own milk as early as possible including subsequent successful lactation, the mother should start pumping and stimulate the breast to milk production as soon as possible after childbirth, preferably during the first 1-6 hours (31). To optimise the prerequisites for adequate milk production the parents should if possible have received appropriate information before birth of the infant.

It is desirable to give optimal support for parents to a preterm infant during the first weeks after birth, as an adequate milk production at two weeks of age after birth is associated with successful breast feeding at discharge from the hospital (32).

Mother's own expressed milk is given fresh or after freezing and defrosting, and in Sweden there is no tradition of heat-treating mother's own milk. Neither sampling for bacteria nor hygiene checks in general is done, unless there is a suspicion of infection. However, you may consider the possibility to take a sample for bacterial culture at least once in the mother of an extremely preterm infant (< 28 gestational weeks) to have a control on mother's own milk within a short period after birth. The milk can be given to the infant without delay of culture results. The above mentioned cut-off points for bacterial counts can be used, but you should also check for MRSA and ESBL-producing bacteria in numbers below cut-off points. If the bacterial counts are above the cut-off points or if MRSA or ESBL-producing bacteria is detected, you should consider pasteurisation of mother's own milk.

For various reasons it may be necessary for the nursing mother to take medications. The breast milk can often be used during such periods, but with certain medications the donation may be suspended. This must be decided individually, depending on the medication, the dosage, etc. (In Sweden, FASS, the Swedish medical products list at www.fass.se is consulted).

Mother's own milk can be given successfully as it is expressed, alternatively with previously frozen milk. However, many hospital units try to give the milk to the infant in chronological order, in other words in the order in which it was expressed. Using this system a reduced variation in nutrient intake is achieved, as the nutritional content of, for example, levels of protein decreases as lactation

progresses. The milk of an individual mother can vary greatly in nutritional content over the course of a 24-hour period, in the amounts of protein, and especially of fat (33-36). By routinely mixing all milk being expressed over 24 hours, this variation can be significantly reduced before the milk is given to the infant or, alternatively, frozen (37,38).

This means that the majority of the milk supplied has been frozen and defrosted previous to use, which can be an advantage, especially from a virological point of view. In recent years there has been an increased interest in the risk of acquired symptomatic postnatal cytomegalovirus (CMV) infection in preterm infants, particularly in extremely preterm infants (26,39). Around seventy percent of all pregnant women in Sweden carry CMV antibodies, with a risk of reactivation of the infection when lactating (40). Thus an infant can be infected by its own mother's breast milk. The risk of a CMV infection appearing as a sepsis-like syndrome is probably very little but the clinical picture can in exceptional cases become serious, especially in very immature infants, necessitating ventilator treatment.

Some studies have indicated that by freezing the milk before use, the risk of breast-milk mediated postnatal CMV infection can be reduced (41,42). This could however not be verified in a new randomized Swedish study (43). Freezing did not affect transmission to extremely preterm infants, but with an overall low risk for transmission of CMV virus (7%). Today there is insufficient scientific support for routinely pasteurising mother's own milk before being given to the preterm infant. However, infants who during the hospital stay suffer from septicemia, cholestasis, or necrotising enterocolitis (NEC) should be investigated for CMV infection (43,44).

From the first day of life, it is important on a regular basis to supply the preterm infant small amounts of fresh colostrum or mother's own milk (drips to 0.5 mL) by mouth, when available. This will among other positive effects stimulate the development of an appropriate mixture of bacteria in the gut (45-47).

Nutritional assessment of mother's own milk

With some local variations, the milk of mothers with preterm infants (<32 weeks gestational age) is nutritionally analysed, from the time the infant is 1-2 weeks old, and subsequently every half - whole week (48). Nutritional analyses can also be made of other breast milk for other reasons.

The milk sample is taken from a 24-hour collection if possible; the milk is collected in the refrigerator for a 24-hour period (preferably from one morning until the following morning – do not include two morning collections!) Nutritional analysis of a random sample of breast milk gives, because of the great variation, a result that is difficult to interpret. It cannot be used as a basis for calculation of the nutritional intake (37,49,50).

A representative sample is taken **after careful mixing** of the 24-hour batch (possibly using a wire whisk to gently mix the fat). Note that this is very important, as there is a great risk that the fat in the milk, especially if it is refrigerated, can adhere to various surfaces that can lead to falsely low fat values.

If the mother has insufficient amounts of her own milk and it is impossible to collect milk over a 24-hour period, taking 1-2 mL of milk from each milk collection can be considered. After storage in the refrigerator, these samples are mixed into a 24-hour collection, which is then analysed. This gives a somewhat less reliable picture of the average nutritional content of the milk, but can be a considerably better alternative than giving donor milk or not analysing the milk at all.

The sample (5-10 mL) should be transferred into a plastic tube; the volume is determined by which equipment is being used. Pour the milk directly into the test tube from the 24-hour collection! Avoid

intermediate steps such as drawing it up into a syringe; every extra step in the milk transfer increases the risk of fat globules adhering to the sides of a vessel, resulting in inaccurately low concentrations of fat and energy.

If the sample cannot be kept refrigerated or is to be sent for analysis to a distant laboratory, 20 µL of bronopol (preservative) shall be added per 10 mL milk. (Note that bronopol is very toxic!) Bronopol is to be kept and used outside the milk kitchen.

Label the tube with: 1. The child's name, 2. Birth date/Personal identity number, and 3. Date of milk collection.

Practical handling of breast milk

Basic rules of hygiene shall be followed in all handling of breast milk.

Freezing

Mother's own milk

Mother's own milk shall be transferred to a plastic container that is closed with a lid. Protect the milk from daylight to avoid degrading of vitamins and reduce the oxidation of fatty acids. The milk is collected in the refrigerator during the day; each new portion of expressed milk shall be chilled separately before it is mixed with other milk. Do not fill the container all the way to the top; leave about 2 cm [about ¾ in] under the lid, as the frozen milk expands. The entire 24-hour collection can be mixed before it is frozen in suitable volumes, to minimize variation in nutritional content, primarily of fat and protein (36-38,49-51).

Always label with: 1. Child's name. 2. Birth date and personal identity number (when there is a risk of mix-up). 3. Expression date. The milk is to be frozen at -20°C. At the hospital the freezer's temperature shall be documented in a standardized manner, and the freezer shall be connected to an alarm when necessary. Freezing breast milk does not affect the bacteria count, or the activity of such enzymes as lipase, or the proteins. However, living cells such as macrophages or lymphocytes are killed (20), which appears to be of little practical significance.

Donor milk

Donor milk shall be kept frozen in plastic containers marked with code numbers and dates of heat treatment. Data on the donated milk (nutritional content, total amount, and where the milk is stored) is kept accessible for the care staff.

Storage

The breast milk is stored in the dark in refrigerators and freezers in the milk kitchen in or adjacent to the neonatal unit. Avoid light exposure by covering the milk with a towel. Light may destroy the vitamins and also affects fatty acids, particularly polyunsaturated fatty acids.

Refrigerator

Fresh and defrosted pasteurised and unpasteurised breast milk can be kept in a refrigerator at +4-6°C for up to 48 hours (52-54). If the milk is to be frozen, this should be done within 24 hours. Newly pumped body temperature milk shall not be mixed with refrigerator-cold or frozen milk, but must be chilled in a separate container first (20). The temperature in the refrigerator at the hospital shall be checked and documented in a standard manner, and the refrigerator shall be connected to an alarm.

Freezer

Breast milk can be kept in a freezer (at least -20°C) for a maximum of 6 months (13). For donated milk this refers to the entire time it is frozen, i.e. the total frozen time before and after heat treatment (16).

Defrosting

Frozen milk shall be defrosted at room temperature, in a water bath with **cold** water fresh from the tap. This should take about 2-3 hours for 100-200 mL. For larger volumes, defrost overnight in a refrigerator.

Defrosting in a microwave oven is not recommended because of uneven temperature distribution (15,21,28,29)! Defrosted breast milk can be kept at room temperature for a maximum of 2 hours before it is given to the infant.

Warming

The milk shall be warmed shortly before feeding.

- **> 20-30 mL:** Warm water bath in a plastic container. Use warm water fresh from the tap. The time can be 10-20 min, possibly longer for larger volumes. Avoid use of bottle warmer, especially if < 100 mL, because of the risk of boiling dry.
- **< 20-30 mL:** Warm water bath in a drug dosage cup with lid or a syringe. Use warm water fresh from the tap. Time up to 5 min. For small volumes, warm under running water.

Always check the milk temperature before feeding. Try to give all milk at body temperature, but never warmer ($32-37^{\circ}\text{C}$). Lower milk temperature causes slower gastric emptying (55).

Pump expression techniques

- Electric pump recommended
- Hand pump possibly
- Hand expression recommended the first few hours after delivery in order to stimulate milk production, but not after established milk production
- "Drip milk" not accepted

In order to start lactation quickly, the mother should start to pump as soon as possible after childbirth, preferably during the first hour after delivery (13,31).

The dominant method is to use an electric pump (56-58). A hand pump can also be used, but it is usually difficult to collect even moderate amounts for a milk bank this way, and can increase the risk of bacterial contamination (21). Hand expression is more dubious because of lower fat content, smaller volumes, as well as an increased risk of bacterial contamination (57) and is not a method to be used by milk donors. It can however stimulate milk production and give access to the first milk drops from the mother. "Drip milk" (milk that spontaneously drips from one breast during nursing or pumping at the other) has a low fat and protein content, and poses a risk for bacterial contamination (20,21,49,51). It shall not be used as donor milk.

It is important that a donor receives information about the various methods available for pumping. The milk production is affected by how many times per day the woman is pumping or breastfeeding. If the infant is not nursing and the electric pump is used to stimulate milk production, the pumping shall be done 6 to 8, possibly even 10 times during a 24-hour period (59). Until the milk production is established, it is often sufficient to pump for 5 min on each breast, and then for as long as the milk is secreted, a maximum of 15 (to 20) min per pumping session. There are different sizes of breast shields to be used with the breast pump, and the mothers should be advised to find the appropriate size.

A few minutes of breast massage before expression enhances the release of the oxytocin and prolactin hormones. When levels of prolactin increase, it leads to increased milk production. Skin-to-skin contact with the infant is also effective during pumping. Double pumping, i.e. pumping both breasts simultaneously, is an efficient way to increase the milk volume because more hormones are being released. It can also save time (60). There are different programs to be installed in the electric breast pump to make expression of the milk more physiological (58,61).

Hygiene rules

To avoid contamination of the banked milk, strict hygiene is required. The donor shall receive written information on the hygiene rules and on how the breast pump's various parts shall be cleaned.

- Approved disposable containers for storage of breast milk are supplied by the milk bank/milk kitchen.
- Hands shall be washed carefully with soap and water and dried with a clean towel. In the hospital disposable paper towels are used, followed by skin disinfectant (alcohol hand gel).
- Both nipple and the areola shall be washed with water, using a disposable paper washcloth. Avoid perfumed soap, which dries the skin and can cause skin irritation.
- Also include the first portion of milk being expressed, as the bacterial count is similar to that of the milk expressed later (62).
- The container used to collect the milk shall be thoroughly washed.

Pumping/nursing room

In the neonatal care unit there shall be a place for the mother to pump milk/nurse her child undisturbed, preferably in the ward room or a special area. If the parents cannot stay with their child on the ward, there shall also be a breast pump available in the overnight room.

Fortification of breast milk

Fortification means adding protein, carbohydrates, fat, minerals and sometimes vitamins to the breast milk (mother's own or donor milk). In Sweden this is done by using a powder product that contains concentrated nutritional elements without adding extra volume to the infant. The preparations available on the Swedish market are all bovine based. There is currently research on human milk-based fortifiers (63) but such preparations are not commercially available in Sweden. Preliminary studies suggest a decreased risk of NEC with human milk-based fortifiers compared to bovine fortifiers (64,65).

Fortification is done mostly for breast milk that is used to feed preterm infants (predominantly < 32 weeks gestational age), and it can be done in different ways:

1. **Individualized fortification**, the method most often used in Sweden today, means that both mother's own and donated milk are enriched according to the nutritional content determined by the nutritional analysis (38,66-69). Nutritional analysis is usually started 7-14 days after birth, and later at ½-1 week intervals (48). One alternative, preferably in combination with milk analyses, can be adjusting the protein fortification using serum concentrations of urea (70,71). These methods are called target(ed) and adjustable fortification, respectively.
2. **Standardized fortification**, also called "blind" fortification. This means that all infants receive milk with identical amounts of fortification, irrespective of what milk is given. This method is inaccurate, and can lead to under- or overnutrition with possible deleterious effects on the infant's central nervous system (72). This method may be used for mother's own milk before the first nutritional analysis but is otherwise not being used in Sweden.

Fortification shall lead to an optimal nutritional intake, and new recommendations for enteral nutritional intakes have been published (73,74).

Regardless of which fortification system is used, there shall be well-developed routines for how the fortification is done in practical terms. It is always safest to weigh out the amount of supplement in a medical cup that at each meal is to be added to a certain volume of breast milk. This cup is kept in the refrigerator during the day. The milk is then enriched before each feeding, because of the risk for bacterial growth in ready-mixed enriched breast milk (75,76). There is also a risk that the carbohydrates in the fortifier will be degraded by the amylase in the milk giving a undesired release of glucose with increased osmolality. This may cause a slower release of the milk from the stomach with a compromised gastrointestinal tolerance as a result (77,78). The fortification powder is also more readily dissolved in the milk if the addition of the preweighed amount of fortifier will take place simultaneously with the warming of the milk before each meal.

When is the optimal time to start fortifying the milk for preterm infants? Previously it was common to wait until the enteral intake could not be increased further. Today, fortification is often initiated at an enteral intake of 50-70 mL per kg per day (38). Some even advocate start of fortification immediately after birth (79). There is also varying opinion about whether to increase the amounts of fortifier subsequently over a few days or supply the total amount at once.

Use electronic scales accurate to at least 0.1 gram. The scales shall be calibrated regularly. Neither enrichment powder nor infant formula powder can be produced in a sterile form as compared to water solutions such as infant formula and some milk fortifiers (not available in Sweden).

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Appendix 1. List of human milk banks in Sweden, 2014

This is a list of the active human milk banks in Sweden, i.e. units that handle breast milk from donors for use by infants other than their own (donated milk).

Hospital	Telephone number	E-Mail-address	No. of donors 2014	No. of liters breast milk 2014
Borås	033-6163129	felicia.johansson@vgregion.se	12	131
Eskilstuna	016-104546	anne.christensen@dll.se	20	84
Falun	023-492033	ulrika.nordfjell@ltdalarna.se malin.hogstrom@ltdalarna.se	88	467
Gävle	026-154124	christina.johansson@regiongavleborg.se	10	93
Göteborg	031-3434265	jannike.fredriksson@vgregion.se lennart.stigson@vgregion.se	101	786
Helsingborg	042-4061937	eva.almeflo@skane.se agneta.elmkvist@skane.se	48	368
Hudiksvall	0650-92318	kristin.norberg@regiongavleborg.se sofie.thoren@regiongavleborg.se	5	45
Jönköping	036-322280	susanne.nilsson@rlj.se	6	70
Kalmar	0480-448179	evy.gustafsson@ltkalmar.se	15	80
Karlskrona	0455-734274	ann-christin.olausson@ltblekinge.se sofie.pollanen@ltblekinge.se	14	60
Karlstad	054-614420	may-britt.larsson@liv.se margareta.billebo@liv.se	51	307
Kristianstad	044-3091830	birgitta.rolandsson@skane.se bo.selander@skane.se	17	165
Linköping	010-1031115	dagny.nilsson@regionostergotland.se	27	155
Lund	046-178438	kirsten.jonsson@skane.se sylvia.markkanen@skane.se	64	394
Malmö	040-331641	marie.a.svensson@skane.se monica.wiberg@skane.se	21	130
Norrköping	010-1043721	gunilla.wastesson@regionostergotland.se josefin.friberg@regionostergotland.se	10	42
Skövde	0500-431928	veronica.norlin@vgregion.se eva.jansson@vgregion.se	41	197
Sunderbyn	0920-282056	annika.m.nilsson@nll.se	5	114
Sundsvall	060-181237	zanita.rytko@lvn.se maria.thelberg@lvn.se	39	335
Stockholm	010-4476356	christiane.engelbretsson@sodersjukhuset.se	142	917
Trollhättan	010-4350340	viktoria.carlsson@vgregion.se	45	750
Umeå	090-7853687	susanne.jacobsson@vll.se inger.ohlund@vll.se	14	203
Uppsala	018-6115878	agnes.pal@akademiska.se	28	348
Västervik	0490-86614	lillian.johansson@ltkalmar.se susanne.adolfsson2@ltkalmar.se	14	80
Västerås	021-173341	carina.olsson@ltv.se ann-sofie.rosenqvist@ltv.se	10	139
Växjö	0470-588010	ulrika.berg@kronoberg.se	25	114
Örebro	019-6021041	gunn-eli.breivik@regionorebralan.se lena.jakobsson2@regionorebralan.se	24	127

Östersund	063-154483	inger.holmberg@jll.se emma.hellner@jll.se	11	130
Total 28			801	5309

Per donor 6.6 liters. (Corresponding numbers for 2009 were 664 donors and 5201 liters)

Appendix 2. Health declaration of breast milk donors (example)

Name

Personal identity number (in Sweden; similar to U.S. Social Security number)

Address

Telephone

Being a breast milk donor carries responsibilities. Your milk will be used to feed small and/or sick newborn infants. We will discuss all the written answers with you, before determining whether you can or cannot donate milk. Circle the appropriate answer below.

Have you donated breast milk before? YES NO

Are you healthy? YES NO

If you answered no, give illness and treatment.

Have you previously had a serious illness that required treatment? YES NO

If you answered yes, give illness and treatment.

Have you had or been exposed to hepatitis? YES NO

Have you been exposed to HIV? YES NO

Have you had a blood transfusion? YES NO

If you answered yes, when and at which hospital?

Have you injected or used narcotics or anabolic steroids? YES NO

Have you had any body piercing or tattooing in the past 12 months? YES NO

Do you drink alcohol? YES NO

If you answered yes, how much per week?

Do you follow any special diet?

YES

NO

If you answered yes, please describe it.

Do you smoke?

YES

NO

Do you use snuff or other tobacco products?

YES

NO

Do you take medications?

YES

NO

If you answered yes, please give type and dose.

In which week of pregnancy was your child born?

Child's birth date

Today's date

Signature

Approved as donor

Signing for milk bank

Physician's signature

Appendix 3. Pasteurisation of donor milk (example)

All handling of milk for heat treatment (pasteurisation) of donor milk shall be done in a separate locale by staff separated from those in care units. The method used is Holder pasteurisation, which involves heating the milk to 62.5°C for 30 min in a Sterifeed Infant Feed Pasteuriser.

Day 1

- Take the breast milk from the freezer the evening before pasteurisation.
- Defrost in the milk containers at room temperature overnight.
- **NB!** The health declaration, the serological screening, and the culture test shall be approved.

Day 2

- Staff shall not start the pasteurisation day with work in the care ward.
- Disinfect the work surfaces and use careful hand hygiene.
- Take out containers and wire whisks.
- Measure out the milk and pour all milk from one donor into a container. If there are several donors use separate containers.
- Label containers to indicate which milk is in each respective one.
- Any possible spills shall be wiped up immediately.
- The milk shall be mixed well; no ice crystals shall be left when the sample is taken for nutritional analysis.
- 5-10 mL milk is poured into a test tube and stored in the refrigerator for individual nutritional analysis or sent to a distant laboratory. Label with code and sample date. Remember to add 20 µL of preservative (bronopol) if the sample is sent to a distant laboratory.
- Label with appropriate code.
- Record in journal the code, when breast milk was collected, and number of liters.
- Codes are given in chronological order and which time in that succession this person has donated milk. For example 16-23B means that the year is 2016, this is the 23rd donor that year, and that this is the second time this donor's milk is being pasteurised.
- Pour the milk into small bottles (130 mL), maximum 120 mL in each bottle. When the milk is expected to have a high protein content (preterm milk), only 50 mL is poured into each bottle.
- Label the bottles carefully, and put them in the rack.
- Start the pasteurisation machine (see separate description).
- When the machine has warmed up, the rack of bottles is placed in the machine.
- When the program is finished the bottles shall be placed in the freezer immediately.
- All material that has been used shall be washed and dried, and all surfaces disinfected.
- When the results of the nutritional analysis have arrived the milk is ready to be used. The results are recorded in a notebook that is kept in the milk kitchen of the neonatal care unit.
- Remember to use the milk in chronological order, i.e. the oldest milk first.
- Counted from the first day of pumping, the milk will keep for 6 months at -20°C.

This information is based on routines used in the Neonatal Clinic in Lund.