

HUMAN MILK BANKING ASSOCIATION OF SOUTH AFRICA



Guidelines for the Operation of a Donor Human Milk Bank in South Africa

Best practice for the collection, storage and
handling of human milk

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1. Introduction

Breastfeeding is best method of infant feeding, because human milk continues to be the only milk which is uniquely suited to the human infant. All mothers should be encouraged to breast feed their own infants. When a mother for some reason is unable to feed her infant, her own milk should be expressed and fed to her infant. Should a mother's own milk be unavailable or insufficient, the next option is to use pasteurised donor milk. For health benefits associated with the use of donor milk see appendix 1.

In 1980 the **World Health Organisation and UNICEF** jointly declared:

“Where it is not possible for the biological mother to breastfeed, the first alternative, if available, should be the use of human milk from other sources. Human milk banks should be made available in appropriate situations.”

The **American Academy of Paediatrics** has stated that Human Milk is superior for infant feeding and is the preferred feeding for all infants, including sick and premature newborns, with rare exceptions. When direct breastfeeding is not possible, expressed human milk should be provided.

The United Kingdom Association for Human Milk Banking www.ukam.org and the Association of North America (HMBANA) www.HMBANA.com have both published guidelines for the establishment and operation of Human Milk Banks.

Much of the information contained in this document has been taken from their guidelines¹⁻³.

These guidelines describe the process of collecting, processing and storing and distributing donated human milk for use in community or hospital settings.

2. Definitions

Human Milk Bank- A donor milk bank is a service established to screen donors, collect, process, store and distribute donated human milk which is used to meet the needs of vulnerable infants in the community or in hospital settings.

Donor Milk- Donor milk is milk given voluntarily, without pay, by healthy lactating mothers for vulnerable infants.

Mother's own milk- Breastmilk, expressed by the mother, for use by her own infant.

Fresh raw milk- Milk expressed and stored in a refrigerator at 4° C for no longer than 24 hours for premature infants and 72 hours for mature infants after expressing.

Fresh frozen milk- Milk which has been expressed and frozen at -18°C for no longer than 3 months from the date of collection.

Pasteurised Milk- Fresh or frozen milk (which has been defrosted) pasteurised by the Holder method at 62.5°C for 30 minutes.

Pooled Milk- Milk from more than one donor that has been mixed together.

Preterm Milk- Milk expressed by the mother of a preterm baby born at or before 36 weeks, and expressed within the first month postpartum.

Processing fees- Fees calculated by the donor milk bank for the cost of collecting screening, processing and distributing donor milk.

Bolus Feeds- When babies are no longer fed continuously but have larger quantities at feeding times.

2.1 Abbreviations

UNICEF- United Nations Children's Fund

HIV- Human Immunodeficiency Virus

CMV- Cytomegalovirus

HTLV- Human Lymphotropic virus

SABR – South African Breastmilk Reserve

3. Administration of Donor Milk Banks (HMBASA)

Donor milk banks should follow procedures and protocols and meet standards set internationally for the administering of donor milk.

In the hospital setting, a Medical Director or head of department should oversee the operation of the Milk Bank. A non-profit organisation in the community should also appoint a Medical Director as advisor.

A Milk Bank Coordinator should be someone with the education, training and experience to plan, develop, implement and evaluate the administrative, medical and educational services of the bank.

Ideally, milk banks should have a panel of consultants from the following areas: neonatology, lactation and microbiology. The panel could also include, where possible consultants from nutrition, public health, and food technology.

4. Records

Donor records are confidential and should include:

- Screening questionnaire
- Serology tests, where applicable
- Date of birth and gestational age of the infant
- A record of each donation made

Administrative records: (see Appendix 7 for labelling)

- A database with date of pasteurisation, number of bottles, donor number in the batch.
- Date of pasteurisation, heat treatment and time
- Bacteriological test results by batch
- Freezer and fridge temperatures
- Milk Bank financial records

Recipient Hospital Records

- Name of doctor/ midwife ordering milk in hospital setting
- Batch numbers of milk supplied
- Where possible outcomes of recipient

Consent of recipient mothers:

Mothers of infants receiving donated breastmilk will need to sign an informed consent form (see Appendix 2).

Indemnity for the hospital (see Appendix 6)

5. Donors

- Donors are healthy women who have undergone screening of lifestyle and have HIV and syphilis test results from pregnancy or a rapid HIV test can be done at the commencement of donation period. Donors are excluded if they do not meet certain criteria; i.e. if they answer yes to any of the questions asked on the screening questionnaire (see Appendix 3).
- If women are on any medication, it is recommended that Thomas Hales' book be used as a resource to check medication compatibility with breastfeeding and the level of risk for infants. A medical advisor should be consulted and the advice is to always err on the side of caution.
- If there is concern about the donor's HIV status, a repeat rapid HIV test should be done every three months.
- Ideally if finance is available, screening of donor mothers for antibodies to hepatitis B and hepatitis C may be required.
- Donors should be mothers who have established lactation and are meeting their own infant's needs and have volunteered to donate surplus breast milk.
- It is suggested that the donor remove all jewellery and her blouse and bra when expressing to reduce potential contamination.

6. Staff

- Staff must adhere to Standards of Practice (SOP) developed by Milk Banks. This should be displayed in a flow chart in the Milk Bank. See Appendix 8 for check list of operation of Milk Banks.
- Human Milk Banking Staff should have regular health checks and be immunised against Hepatitis B.
- Staff must carefully wash their hands with soap and water, brush their finger nails and rub their hands with 70% alcohol for 30 seconds to reduce bacterial load after drying. They should wear hospital gowns, masks and use gloves.
- Staff with long hair should tie hair back.
- Staff should have received training in breastfeeding support and promotion and human milk banking.

7. Own Mother's Milk

- In order to establish a good supply of milk, **if a baby is not breastfeeding**, a mother should start expressing as soon as possible after birth. Thereafter, express milk at least 8-12 times in 24 hours. This is particularly important in the first two weeks to help establish lactation. Double pumping is most effective.
*This stimulates milk production when mothers are expressing for their sick infants.*⁴
- If milk is being expressed for a mother's own baby, this should be used as soon as possible so the infant receives the greatest benefit from the milk.
- Testing of own mother's milk is expensive and results are rarely clinically relevant.⁵
- There is no evidence to support the heat treatment of a mother's own milk which is fed to her child (unless she is HIV infected). *Raw breast milk has sufficient bactericidal and bacteriostatic systems which inhibit the growth of microorganisms in the milk.*
- *Two studies have found no adverse effects in premature babies who ingested bacteria in mother's own milk.*^{6,7}
- *More recent studies have shown the beneficial effects of low birth weight premature infants receiving mother's untreated breast milk.*^{7,8}

8. Collection of Human Milk

- Collection of milk can be performed by hand expression or with a manual or electric breast pump.
- All breastfeeding mothers should be taught about breast massage and hand expressing. *Research has shown that hand expressed milk is less likely to be contaminated⁹ and it also stimulates milk production.*¹⁰
- Mothers should be given written and verbal guidelines on expressing, handling and storage of breast milk and sterilising breast pumps (See Appendix 5).
- Mothers should observe good hygiene. They should not wash their breasts with bactericidal or ordinary soaps, other than their daily shower or bath. *Soap can dry out the breast and lead to cracked nipples*

- Rigorous hand washing with soap and water and drying on clean or disposable towel is recommended.
- Mothers expressing at home should use a quiet place, avoiding areas where contamination could occur e.g. bathrooms and rooms with pets.
- Breast pump used should be disassembled and rinsed with cold water to remove milk. The parts should then be washed in warm soapy water and rinsed. Alternatively, the rinsed pieces can be washed in the dish washer. *Pittard et al found that there was no difference in bacterial contamination of pumped milk when comparing a mother's use of her own clean versus sterile kit¹¹.*
- Sterilization of breast pump parts in the hospital setting is recommended.
- There should be no sharing of breast pumps, purchasing of second hand pumps as this can lead to contamination of the breast milk, the mother or the infant if viruses have not been destroyed by sterilisation.¹²
- Bulb type pumps are strongly discouraged as they carry a high risk of contamination.
- Electric pumps where the motor is not sealed are for individual use only. They should not be shared or bought second hand or hired.
- Only electric breast pumps with an isolated motor, which does not connect with the tubing, are suitable for multiple use. Here each mother should have her own set of tubing. (Medela Lactina)
- Donors should be supplied with labelled, sterilized bottles. Or the donor mother uses a permanent marker (Masking tape tends to come off when the bottle gets wet during pasteurisation) to mark the bottle before use with her donor number and the date of expressing.
- Preferably, only containers provided by the milk bank should be used.
- Expressed milk can be stored in:
 - Glass jars
 - Hard plastic containers made from polypropylene or polycarbonate
 - Standard baby bottles, either glass or plastic
 - All containers must have tight fitting lids
 - Disposable milk bags are not recommended as levels of secretory IgA and lipids are significantly decreased. They also tear easily, are difficult to handle and there is an increased risk of contamination.

- All containers must be labelled with donor mother's number, date of expressing, date of freezing & date of pasteurisation
- Containers should not be filled as milk expands when frozen.
- Milk should be chilled before it is added to frozen milk. For sick or preterm babies it is advisable to use a new container for each pumping.

9. Storage of Milk

- Fresh raw milk can be kept safely at room temperature (25° C) for up to 6 hours. *No bacterial growth occurs at room temperature for up to 6 hours.*¹³
- Fresh raw milk can be stored in a refrigerator at 4° C for 48 hours. *Milk can be kept in a fridge from 2-8 days without an increase in the bacterial count. However because a fridge maybe opened frequently, and the temperature of milk may increase, the safe recommended time is 48 hours.*¹⁴

Donor milk should be refrigerated as soon as possible after it has been expressed, this prevents bacteria multiplication and lipolysis. *Bacterial growth has been shown not to increase at room temperature for up to 6 hours.*¹³

- Milk can be frozen immediately and then more added to the same container over 24 hours, provided that the fresh milk is well chilled. This is not recommended for preterm infants.
- Donor milk should be chilled then frozen as soon as possible. *This preserves Vitamin C content, prevents lipid peroxidation and removes viable Cytomegalovirus(CMV)*¹⁵⁻¹⁷.
- Raw donor milk can be stored in a freezer (-18°C) for a maximum of 3 months **before it is pasteurised**. It should not be stored for longer than this. *Lipolysis can occur in frozen milk after 3 months of storage at -18° C.*¹⁸
- Rigorous temperature control using a thermometer should be undertaken. If temperature fluctuations occur then milk should be used immediately. In South Africa with power cuts this may apply.
- Donor milk stored in the ice box of a fridge should be transferred into a freezer within 1 week. Bottles/containers should be in a separate holder/container to prevent contamination. *Temperature of the ice box is only -4° C.*

- Thawed milk, fresh or pasteurised should be refrigerated after thawing and used within 24 hours.
*Thawed pasteurised milk needs greater protection as it has a slightly decreased ability to inhibit bacteria.*¹⁹
- Thawed milk left at room temperature should be used within 4 hours due to the danger of bacterial growth.
- Human Milk must be stored in a separate container in the freezer at the donors' home.
- At the Milk Bank, human milk must be stored in a separate freezer where the temperature is rigorously controlled. Frozen raw milk should be kept separately from pasteurised milk.
- Milk should not be stored frozen for longer than **6 months from date of expression.**

Table 1. Storage of Human Milk

Human milk	Room temperature 25° C or lower	Refrigerator 4° C	Freezer -18° C
Fresh raw milk	6 hours ¹³	48 hrs ¹⁴	3 mths for prems is best. ¹⁸ 6 mths for term infants
Thawed milk(not heat treated)	<4 hrs	<24 hrs	Do not refreeze
Frozen pasteurised milk	< 4 hrs and discard after feeding	<24 hrs ¹⁹	Do not refreeze

9.1 Bottles – there are several options:

There are 2 recommended types of containers - plastic or glass bottles:

- If glass bottles used then check for chipping. They can be re-used but have to be well washed and sterilised or washed on high temperature cycle in a bottle washer or dishwasher.
- For preterm infants sterilise bottles in autoclave.
- If plastic bottles are used, they should be made from polypropylene (cloudy, hard plastic). The disadvantage of plastic is that there is some immunoglobulin loss due to absorption onto the plastic.
- If plastic bottles are used they should be used once only and discarded. Ensure that the lids seal well.
- For hospital based milk collection then the recommended plastic bottles are Made by Sterifeed. Contact details: Tel: 021 5570558 Fax: 021 5574795 Cell 076 223 1824. E-mail: ken@healthcaretechnologies.co.za Website: www.healthcaretechnologies.co.za

10. Thawing of milk

- Frozen milk should ideally be thawed in a fridge. Milk thawed at room temperature can easily be forgotten. It should not be left at room temperature for longer than 2 hours before using.
- If it is thawed at room temperature, this should be done in the quickest time using a container of warm water (not more than 37° C) making sure that the water does not touch the lid.
There is a danger of water seeping under the lid and contaminating the milk.
- Once milk is thawed but still chilled, it should be refrigerated or pasteurised.
- Thawed milk should not be left at room temperature.
- Never microwave human milk to defrost or warm it.
Microwave thawing decreases activity of lysozyme and IgA. There are also loss of nutrients e.g. Vitamin C, from excessive heat and hot spots from unevenly heated milk.^{20,21}
- Thawed pasteurised milk should not be refrozen, it increases hydrolysis of lipids and increases risk of contamination.

11. Transportation of milk

- Container used must be insulated, rigid and easily cleaned and disinfected.
- Coolant blocks should be used and the empty space in the container should be filled with bubble wrap.
- Milk should be shipped or transported over long distances at -20° C.

12. Pasteurisation

Ensure that the milk is pasteurised as soon after collection. *This preserves Vitamin C content, prevents lipid peroxidation and removes viable Cytomegalovirus(CMV).*¹⁵⁻¹⁷

- Holder method of pasteurisation for 30 min. at 62.5° C; inactivates, CMV, HIV, HTLV and kills most pathogenic bacteria found in breastmilk.²²⁻²⁴

It is preferable to purchase a pasteuriser with a cooling cycle attached to it. Sterifeed manufacture a commercial pasteuriser which digitally tracks each pasteurisation batch recording temperatures and times. It also includes a cooling cycle. Contact details: Tel: 021 5570558 Fax: 021 5574795 Cell: 076 223 1824. E-mail: ken@healthcaretechnologies.co.za
Website: www.healthcaretechnologies.co.za

If the method of pasteurisation is Flash Heating then the milk must be flash heated as soon as possible after it is expressed and should not be frozen, thawed and then flash heated.

13. Bacteriologic Screening of Milk

- This should be done on an aliquot of milk from the first donation of each donor mother. It is done post-pasteurisation and tested for microbial contamination.
- If any contamination is found, milk must be discarded and milk from the donor mother must be rechecked with the subsequent donation.
- For **community based** banks a simpler test which is used by all the Brazilian Breastmilk Banks is known as Titratable Acidity (See Appendix 4).

14. Nutritional Assessment

For hospital based milk banks which are feeding low birth weight infants it is preferable to know the exact protein and energy content of the donor milk. A very simple instrument is available which measures fat, protein, lactose and energy content. The instrument is known as the Miris Human Milk Analyzer and is available from Miris AB in Sweden (info@miris.se; www.miris.se). The cost is approximately 12,000 Euros.

15. Use of Donor milk in the Hospital Setting

- Preferably match gestational age of recipient baby with gestational age of donor mother
- Milk should be completely thawed and gently agitated to mix milk before use to ensure that there is a balance of all the nutrients.
- Continuous tube feeding: Tubing between the pump and tube should be changed 4 hourly.
*Bacteria can multiply in the infusion syringe and tubing.*²⁵
- The shortest possible tubing should be used and the syringe tip should be tipped upwards to maximize the amount of fat delivered during the feed.
Fat sticks to the side of tubing and of the syringe and less fat is lost if the syringe is tilted upwards.^{26,27}
- Less fat is lost with intermittent bolus feeding.²⁸
- Cup feeding can be used for an infant from about 30 weeks gestation once 2-3 hourly bolus feeds have been established.
- Bottle feeding is not recommended as it can interfere with the establishment of breast feeding.

16. Flash heating

- Where there is no access to pasteurisers, milk can be pasteurised using the following method:
- Express up to 120ml of milk into a clean 450-500ml glass jar, screw lid on until ready for flash heating.
- When ready to flash heat remove lid, place in a 1 litre aluminium Hart pot and add **cold water** sufficient to cover the level of milk by 2 fingers widths.

- Place the pot containing water and jar in the middle of heat source.
- Wait until the water reaches a **rolling boil** and remove the jar with the breastmilk and allow to cool.
- If the process is repeated, add **cold water** to the pot to flash heat more milk.
- Mothers wanting to heat treat **their own milk**, should preferably flash heat it as soon after expression as possible and then it can be stored for 8 hours at room temperature.

Heat treatment destroys HIV and any possible contamination²⁹

17. Governance

All banks will operate autonomously however they will fall under the regulatory control of the Human Milk Banking Association of South Africa (HMBASA).

All banks must endeavour to:

- Include activities which protect, promote and support breastfeeding
- To comply with draft regulations relating to foodstuffs for infants and young children (No 30402)
- Include a lactation consultant on their committee.

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Appendix 1

Potential Advantages of Human Milk

(Taken from the Paediatric Policy of the Royal Australian College of Physicians)

Known nutritional advantages of HM are related to the proteins, fats, growth factors, hormones, enzymes, anti-oxidants and other bioactive components of human milk, and the bio-availability of trace elements, vitamins and minerals.

Although the quantity of protein in HM is less than in AF, the protein quality and bio-availability is superior. Unique bioactive proteins include alpha-lactalbumin, beta-lactoglobulin, lactoferrin, lysozyme, serum albumin, IgA, IgG and IgM. Breastmilk also contains hormonally active peptides [\(1\)](#) and nucleotides [\(2\)](#).

The fat composition of HM is also unique and is reflected in brain growth and myelination [\(3\)](#). All HM and some AF contain long chain polyunsaturated fatty acids (LCPUFAs), and there is some evidence that these are important for early retinal and visual development [\(4\)](#).

HM carbohydrates are also unique. HM contains more than 130 oligosaccharides which may contribute to brain maturation and host defence [\(5\)](#).

The risk of necrotising enterocolitis (NEC) in pre-term infants is increased when AF is fed rather than HM [\(6\)](#), and this difference is most marked when AF infants are compared with infants fed fresh or unprocessed mothers' milk. There is also evidence of better feed tolerance and quicker establishment of full enteral feeds in pre-term infants fed HM compared to infants fed AF [\(7\)](#).

There is some evidence for a reduced incidence of allergy in infants of families with a history of atopy if the infants are fed only HM for some months [\(8,9\)](#).

HM provides additional immunological and cellular benefits. Pasteurisation retains most of the IgA, lysozyme and lactoferrin, although pasteurisation and/or freezing destroys the cellular elements [\(10\)](#). The risk of infection is increased for infants fed AF rather than HM [\(11\)](#).

Feeding AF may be associated with higher rates of type 1 diabetes in later life [\(12\)](#).

There is a suggestion that pre-term infants fed their mothers' own milk may have been shown to have an IQ advantage at age 8 when compared with those fed AF [\(13\)](#).

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Appendix 2 Consent for Donor Milk

Human Milk provides the best nutrition for infants and young children it promotes normal growth and development and helps to reduce the risk of illness. Human milk has a unique composition of nutrients, enzymes, growth factors, anti-inflammatory and immune properties which have never been replicated. The best option for a baby is its mothers own milk. When this is not available, the next best option is pasteurised donor milk from a milk bank. Pasteurised milk retains most of the nutrients and properties which protect the baby from infections.

The Milk Bank follows strict guidelines to ensure a safest possible product. The donors are healthy mothers who have been screened and the milk is pasteurized and checked for bacterial growth.

I understand the above information and I(legal guardian)
give consent for my baby.....to receive
pasteurised human donor milk.

Date: _____

Witness: _____

Appendix 3
Breastmilk Bank Screening Questionnaire

Donor's Details

Donor No. _____

Name: _____

Contact Details: _____

Baby's birth date: _____

This information will be treated as confidential

#	Question	Reply
1.	Have you received a blood transfusion or blood products in the last 12 months?	
2.	Do you regularly have more than 50ml of hard liquor or its equivalent in a 24-hour period?	
3.	Regular use of medications, or use of radio-active drugs or cytotoxins?	
4.	Are you a total vegetarian?	
5.	If yes, do you supplement your diet with B12 vitamins?	
6.	Do you use habit-forming drugs?	
7.	Do you smoke?	
8.	Have you ever had hepatitis B, HIV, or TB?	
9.	Have you ever had a sexual partner who is at risk for HIV, takes habit-forming drugs, or is a hemophiliac?	
10.	Do you have a copy of the results of your anti-natal HIV tests?	
11.	If not, would you be prepared to undergo a rapid test for HIV at your expense and submit the results to the screening officer?	
12.	Have you had a piercing of any part of your body or a tattoo in the past year? If yes can you guarantee that the stud used to pierce you remained in situ?	
13.	Have you had an MMR vaccine in the past month?	

Appendix 4

Methods for determining safety of milk

4A Titratable Acidity

1. PRINCIPLE

A certain amount of milk is titrated with 0.1N sodium hydroxide in the presence of phenolphthalein as indicator. Phenolphthalein is colourless in an acid medium but turns pink in an alkaline solution. The acidity of fresh milk is mainly due to the presence of proteins, phosphates, carbon dioxide and citrates and is called inherent or natural acidity. Any increase in acidity is due to the conversion of lactose to lactic acid by the action of bacteria. This acidity is called developed or real acidity. In practical work the developed acidity is not distinguished from the inherent and the total acidity is expressed as % lactic acid. This test is done post pasteurisation before the breast milk is used.

2. APPARATUS and REAGENTS

ITEM	SUPPLIER
Burette (10ml or 25ml) connected to supply bottle	Merck
White cup or 50ml glass beaker	Merck
White background	--
Bulb pipette (9ml)	Merck
Aqueous Sodium Hydroxide 0.1N	Merck
Phenolphthalein 1% solution in 95% alcohol Preferably in a dropping bottle	Merck

3. PROCEDURE

- 3.1 Pipette 9ml of the well mixed sample into the cup or beaker.
- 3.2 Add 4 – 5 drops of phenolphthalein indicator.
- 3.3 Titrate with 0.1N sodium hydroxide until the first definite and relatively permanent shade of pink is obtained (Note 4.3, 4.4 and 4.5).
- 3.4 Read the burette to determine the ml of sodium hydroxide used.

3.5 Multiply this reading by 0.1 to obtain the result expressed as % lactic acid.

4. NOTES

4.1 The aqueous sodium hydroxide solution must be protected from the air as much as possible as it reacts with carbon dioxide which weakens the solution. The stock bottle should be small and should be kept tightly stopped. It is advisable to protect the contents of the burette against contact with carbon dioxide by means of a drying tube supplied with calcium oxide. Otherwise, unused solution in the burette should be discarded at the end of each day.

4.2 The strength of the titres of the caustic soda solution must be checked against the 0.1N sulphuric acid at least once per week. If the titre is not within 0.098 – 0.102, it must be adjusted by addition of 1N sodium hydroxide or distilled water.

4.3 The titration should always be carried out in good light to enable the first colour change to be detected. Daylight is preferred to artificial light.

4.4 The titration should be carried out fairly rapidly as slow titration yields higher results.

4.5 During titrations, the temperature of the sample and reagents should fall within the range 15 – 25 °C.

4B The Phosphatase Test

1. PRINCIPLE

The Phosphatase test is applied to milk (or cream) to determine whether pasteurization was done properly and also detect the possible addition of raw milk to pasteurized milk. Hence the phosphatase test is done directly after pasteurisation once the sample has cooled down. Phosphatase is a natural milk enzyme which is inactivated when milk is pasteurized. The Aschaffenburg-Mullen phosphatase test is based on the principle that the phosphatase enzyme present in raw or underpasteurised milk hydrolyses the substrate p-nitrophenylphosphate in alkaline solution (colourless) with formation of p-nitrophenol. The latter compound is yellow and serves as a direct indicator of residual phosphatase activity.

2. EQUIPMENT

2.1 Lovibond comparator with disc APTW5, APTW7 or 4/13

2.2 Water bath at $37 \pm 0.5^\circ\text{C}$

2.3 Pipettes, 1ml and 5ml

2.4 Volumetric flasks, 100ml and 1L

2.5 Test tubes, nominal size 150 x 16 mm with rubber stoppers or Cap-O-Test closures

All glassware must be carefully washed with hot water containing sodium carbonate, rinsed with warm water, rinsed with distilled water and finally dried. If the glassware does not appear to be clean after this treatment, the glassware should be soaked in dilute hydrochloric acid, rinsed with warm water, rinsed with distilled water and dried. Glassware used for this test should not be used for any other purpose and should be stored under dust free conditions. New rubber stoppers should be boiled repeatedly in distilled water and dried. Rubber stoppers should always be boiled in distilled water after use.

3. REAGENTS

3.1 Buffer solution: Dissolve 3.5g anhydrous sodium carbonate (Na_2CO_3 , AR or GR grade) and 1.5g sodium bicarbonate (NaHCO_3 , AR or GR grade) in distilled water and make it up to 1 litre in a volumetric flask. This solution can be kept for 3 months.

3.2 Substrate: Disodium p-nitrophenyl phosphate hexahydrate (Merck No 106850 or Fluka No. 71770). This reagent must be stored in a refrigerator. Take container out of refrigerator approx. 1 hour before opening.

3.3 Buffer – substrate solution: Accurately weigh 150mg of substrate (3.2) into a 100ml volumetric flask and make up to the mark with buffer solution (3.1). Shake to dissolve and mix properly. The solution should be very pale yellow and should give a reading of not more than 10 on the phosphatase disc when viewed in the Lovibond comparator, using a blank of distilled water on the left-hand side. The solution must be kept in a refrigerator and used for a maximum of one week.

4. SAMPLE HANDLING AND STORAGE

4.1 Samples should be tested as soon as possible after receipt in the laboratory.

4.2 If a sample is not tested immediately, it must be kept in a refrigerator or on ice until testing. The proper cooling of samples until immediately before testing is very important as reactivation of phosphatase might otherwise occur leading to false-positive results.

4.3 Samples which show evidence of taint or souring should not be tested.

5. PROCEDURE

5.1 Transfer 5ml of the buffer-substrate solution to a test tube, close tube, and bring to a temperature of 37°C in a water bath. When more than 1 sample is tested, the requisite number of tubes must be prepared in this way.

5.2 Add 1ml of the sample to be tested, mix properly, and incubate at 37°C for 30 min. For practical purposes, the sample may be added before preheating the buffer-substrate solution. In this case the incubation time must be extended to 33 min. to allow for the time it takes for the tube contents to reach a temperature of 37°C .

5.3 With each sample or series of samples, incubate one blank prepared from milk (cream when testing cream samples) which has been boiled or heated in a water bath at $75\pm 2^{\circ}\text{C}$ for 3 min.

5.4 Remove the tubes from the water bath and mix the contents by inverting the tubes several times.

5.5 Place the blank in the left hand opening and the sample in the right-hand opening of the comparator.

5.6 Place or hold the comparator facing a good source of daylight (or use a white light cabinet), but avoid direct sunlight.

5.7 Revolve the disc until the sample colour is matched and record the reading.

5.8 A positive control using 0.3ml of raw milk in 100ml of pasteurized milk must be carried out at least once per week, preferably after a fresh buffer-substrate solution has been made up (this must give a reading of at least 14 after 30 mins).

6. INTERPRETATION OF RESULTS

Disc reading (30 min.)

0	Properly pasteurized
6	Doubtful
≥ 10	Underpasteurised

Samples giving a reading of 6 must be replaced in the water bath for a further 90 min. incubation. The 2 hour test result is interpreted as follows:

Disc reading (2 hours)

0 to 10	Properly Pasteurized
>10 to 18	Slightly underpasteurized
>18 to 42	Underpasteurized
>42	Grossly underpasteurized

7. REFERENCES

The determination of phosphatase in milk using the Aschaffenburg and Mullen Phosphatase Test, Colorimetric Chemical Analytical Methods p. 603-5

Government Gazette, Vol 389, No. 18439, Regulation No. R1555 (1997), p.23-4.

8. EQUIPMENT AND CONSUMMABLES LIST

ITEM	SUPPLIER
Lovibond Comparator with disc APTW5, APTW7 or 4/13	Merck / Lasec
Memmert Waterbath (37°C)	Labfix
Pipettes (1ml and 5ml)	Merck
Test Tubes (150 x 16 mm)	Merck
Cap-O-Test	Merck
Volumetric flasks (1L and 100ml)	Merck
Anhydrous Sodium Carbonate (AR or GR grade)	Merck
Sodium Bicarbonate (AR or GR grade)	Merck
Disodium p-nitrophenyl phosphate hexahydrate (Merck No. 106850 or Fluka No. 71770)	Merck

4C Microbiological Cultures

4C.1 3M Petrifilm

DESCRIPTION

3M Petrifilm *E.coli*/Coliform Count Plate (EC) is a ready made culture medium system which contains Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity (5-bromo-5-chloro-3-indolyl β -D-glucuronide (BCIG), and a tetrzolium indicator that facilitates colony enumeration. Petrifilm EC plates are useful for the enumeration of *E. coli* and coliform bacteria in the food and dairy industries and are decontaminated though not sterilised. This Petrifilm EC test is done post pasteurization to check for bacterial contamination in each batch.

METHOD

Supply 1ml of pasteurized breast milk in a sterile container and send it to a microbiology laboratory for the enumeration of *E.coli* and coliform colonies on Petifilm EC plates.

4C.1 McConkey Agar Plate Cultures

DESCRIPTION

This method is ideal for breastmilk banks in hospitals which have a microbiology lab that does routine microbiological screening of samples. This method is often used on random screening of formula milk prepared in the milk kitchens of neonatal nurseries.

METHOD

Samples of milk will be put into sterile containers and transported on ice to laboratory within 4 hours. If there are any delays with transport, the milk should be refrigerated. 100 μ l of each sample will be plated onto MacConkey agar and incubated for 24hrs at 37⁰C. The following day a semi-quantitative count will be performed. Only if required, will any suspicious colonies be further identified.

Appendix 5

Instructions to Donor Mothers

Expressing and storing your breast milk

1. As long as you bath everyday you do not have to wash your breast before you express. Please do wash your hands very carefully with soap and running water before expressing milk.
2. Always use a sterile bottle to collect your milk and mark the bottle with your donor number and the date. Seal the lid as soon as possible after expressing.
3. Newly collected milk should be chilled immediately, either in the freezer straight away or in the fridge first and then frozen at the end of the day. If the bottle is not full, you can add newly collected milk to the previously stored milk on the same day, as long as the milk is chilled first.
4. Store the bottles of milk in a separate container (box or plastic bag) in the freezer, preferably at the back or near the bottom of the freezer.
5. When you are expressing sit somewhere comfortable, relax have your baby close by and if necessary warm your breasts. You can gently massage your whole breast by rolling using your knuckles working from the outside towards your nipple. Twiddle your nipples to stimulate them, and then either express using a breast pump or using your hands.
6. Remember not to overfill the bottles leave a 2 cm gap at the top of the bottle.

Remember: When you first start expressing you may only get a few drops of milk. This improves with practice and as you do it more regularly. Try expressing at the same time every day. Many mothers find the mornings are a good time to express milk.

Sterilising the pump

All parts of the breast pump must be sterilized before use. The breast pump must be washed rinsed in cool water and then washed and rinsed in hot soapy water before being sterilized. Follow the manufacturer's instructions for sterilizing.

Appendix 6: Indemnity for Hospitals

Indemnity Agreement between..... (Milk Bank)
and.....

When donor mothers are identified and screened by(Milk Bank)
it is the responsibility of(Milk Bank) to educate these donor
mothers regarding safe expressing, freezing and transportation of their
breastmilk.

When donor mothers are identified and screened by
it is the aforementioned hospital's responsibility to educate donor mothers
regarding safe expressing, freezing and transportation of their breastmilk.

.....(Milk Bank) takes the responsibility to pasteurise,
rapidly cool and freeze (time permitting) the donor breastmilk, before the
pasteurised milk is issued to.....

It is the responsibility of any hospital/institution receiving pasteurised milk
from(Milk Bank) to verify such tests, do further
tests if required and maintain adequate records. It is the responsibility of the
hospital/institution who is to administer the pasteurised milk, to obtain
signed consent from the recipient parent/caregiver or guardian.

.....(Milk Bank) therefore indemnifies itself and all
associates from any complications arising from contamination, handling or
processing of the milk that occurs before and after it has been pasteurised
by.....

I,, from (Hospital/Institution) in the capacity of
..... agree to indemnify.....(Milk Bank) as
stated above.

Date _____ Signature _____

Date _____ Witness _____

Appendix 7
7.1 Labeling

Mother fills in the underlined section, the rest is completed by Milk Bank Staff

<u>Donor number:</u>	<i>Use Before:</i>
<u>Expressing date:</u> /..... /.....	<u>Freezing date:</u> /..... /.....
Pasteurisation date: /...../.....	Batch no:

Recipient name:	
Number:	
Thawing date: /..... /.....	Time of thawing: h
Expiry date: / /.....	Expiry time: h
Store for less than 24 hours after thawing	

7.2 Donor Milk Order Form

We support Exclusive Breast Feeding (EBF) and Exclusive Breast Milk Feeding (EBMF). Own Mother's Milk (OMM) is first choice (fresh or pasteurised) for infant feeding.

Donor Expressed Breast Milk (DEBM) may only be ordered if:

Mother's criteria

- Mother is seriously ill
- Mother building up own supply – 1st three days post partum
- Mother is absent – unable to visit baby and no rooming-in bed available
- Other maternal contraindication - chemotherapy, serious substance abuse, etc

AND

Baby's criteria

- Preterm or ill babies of HIV positive mothers.
- Preterm babies < 1500g
- Resumption of feeds for babies with 'abdominal mischief'

Mother / guardian signs consent for DEBM

A copy of the consent form must accompany first orders.

Donor Milk stocks are finite and it may not always be possible to meet every order.

.....(Milk Bank) will endeavour to supply DEBM until eligible babies reach 2 weeks of age. Beyond this age, orders for DEBM require special motivation.

Please note that defrosted DEBM must be discarded 4 hours after thawing if kept at room temperature and after 24 hours if kept refrigerated at 4°C.

Date:/...../.....

<u>Sticker or Name, folder number, DOB</u>	<u>Location (eg MMH ICU 7)</u>	<u>Gestation /B.Wt</u>	<u>Maternal Retrostatus</u>	<u>Indication for DEBM</u>

Date: /.... /.... **Daily Order form for DEBM**

Please supply DEBM for these babies. Signed:
(Sr/Dr Name:)

<u>Name</u>	<u>Folder Number</u>	<u>Location</u>	<u>Feed number</u>	<u>Age in days</u>	<u>Gestation</u>

NB: Copy of consent form to be sent to milk bank if baby is getting DEBM for first time.

Appendix 8

Check list for operation of Human Milk Banks registered with HMBASA

This checklist is based on the NHS National Institute for Health and Clinical Excellence guidelines. (February 2010)

1. Purpose

- To ensure consistent conformance and quality of breast milk that is heat treated.
- To minimize the variation and ensure a safe end product even with personnel changes.
- Minimize opportunity for miscommunication
- Address safety concerns
- Improve comparability, credibility and legal defensibility
- To ensure that all Human Milk Banks operating in South Africa are adhering to HMBASA Guidelines.

2. Summary

Quality Assurance- Hazard Analysis and Critical Point (HACCP) principles should be the guideline in all processes. Equipment is to be serviced, maintained, calibrated and recorded. Training of staff on a regular basis to ensure competence in all aspects of the operation of Milk Bank. This includes health and safety, all operation of equipment record keeping and related ethical and legal matters.

Administration of Milk Banks- qualified staff

Screening of donors- follow screening process

Training of Donors-face-to-face training to ensure safe product

Donor Mother's handling of milk- Immediate freezing to preserve product

Handling of milk at Milk Bank- Safe practice for transport, storage and pasteurisation

Tracking and tracing- Labeling and record keeping

3. Quality assurance

Clean, sterilize and store all containers according to protocols	
Calibrate and inspect equipment used for pasteurization. Follow manufacturer's guidelines	
Check water temperature of pasteuriser using a thermometer at 3 monthly intervals	
On-going training of staff to cover: Good practice Technical know how Health and safety issues Pasteurisation and food hygiene Regulatory, ethical and legal aspects of job	
Administer quality control system: Screening, collection, transportation storage of milk	

4. Administration of Donor Milk Banks

In hospital setting, Medical Director or HOD should oversee operation of milk Bank	
Milk Bank coordinator- education and training in lactation	
Panel of consultants: neonatology, lactation and microbiology	

5. Screening Donors

Check donor mothers health	
Age, date and health of baby	
Smoking	
Any medication	
Recent exposure to infection HIV 1 or 2 Hep, B or C, HTVL I or II, syphilis, herpes or chronic infection	
Results of HIV and syphilis test	
Consent from Donor Mother to use her milk	

6. Training donors

Meet mothers and train face-to-face	
Training to cover: Good hygiene, including hand washing Expressing and collection of milk Sterilisation of breast pumps and containers No sharing of breast pumps or use of sterilised second hand pumps Storage of breast milk	
On-going support and advice for mothers on breastfeeding	
Advise donor mothers to contact the milk bank should they develop any infection or start any medication	

7. Donor mother's handling of donor milk

Freeze milk immediately after expressing.	
Check freezer is working correctly	
Containers supplied by Milk Bank must be used	
Milk transported should be done so in secure cool boxes with dry ice	

8. Handling of donor milk at the Milk Bank

All processing must occur under hygienic conditions. Good hand washing.	
Wearing sterile gloves when handling milk	
Labelling: Donor number, date of expression and date of pasteurisation on each bottle	
Store pasteurised and unpasteurised milk in separate freezers	
Store at - 20° C	
Store unpasteurised milk for no longer than 3 months from date of expression	

before pasteurisation	
Store thawed donor milk for no longer than 24 hrs in a fridge	
Prevent thawed milk from reaching 8°C prior to pasteurisation	
Pasteurise milk at 62.5° C for 30 min	
Following pasteurisation, rapidly cool milk to 4°C or lower within 10 minutes	
Store frozen donor milk for no longer than 6 months from date of expression	
Regularly test donor milk for microbial contamination (once a month or every 10 cycles whichever comes first)	

9. Tracking and tracing

Track donor milk from donor to recipient	
Monitoring should include freezer temperatures, pasteurisation processes and stock control	
Records of donor should include: donor number, consent, screening questionnaire, results of tests.	
For each pasteurised batch: keep records of batch number, date of pasteurisation, donor numbers pasteurised, microbiology samples taken, and recipients who receive milk.	
Labelling: Donor number, date of expression, pasteurisation date, instructions to keep frozen and use within 24 hrs if defrosted, and expiry date.	
Receiving unit should keep records of: Baby's name, batch number, donor number and date, consent of recipient mother	