The Storage of Breast Milk

A review written for MAM Babyartikel GesmbH

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Summary, Conclusions and Recommendations

At the request of MAM Babyartikel GesmbH we have reviewed the literature regarding the storage of expressed breast milk to assist in the design of a new storage system. In the main this report confines itself to the question of breast milk and healthy children.

The benefits of breast feeding and of using expressed milk are briefly reviewed. Undoubtedly the use of expressed breast milk is the next best thing to the breast itself.

The effect of storage times and temperature are analysed with particular emphasis on the essential constituents of breast milk and on bacterial contamination. Clearly, expressed milk begins to deteriorate as soon as it leaves the breast. However, within reason, these changes are not significant for healthy children.

There is a wide disparity in both research findings and recommendations for "safe" storage. From the data available a recommendation has been proposed (room temperature 4 hours; fridge (4^{o}C) 3 days; freezer (-20^{o}C) 3 months). In addition, the need the emphasise cleanliness in any instruction literature is outlined.

The effect of different materials of constructions on milk quality is reviewed. Good quality data is somewhat limited. However the suitability of polyethylene (particularly) and polypropylene has been confirmed and a number of recommendations have been made, particularly in regard to conforming to food contact regulations and pre-sterilisation.

A brief survey of storage systems currently on the market has been undertaken and a number of examples are given.

The information given in the instruction leaflet and other literature will be a key element of any product and a number of recommendations are outlined.

The storage of breast milk

Background

MAM are developing a system for the storage and use of expressed breast milk centred on the Ultivent bottle.

They requested a review of the literature to assist their design process and also to provide guidelines regarding the best conditions of storage. In particular we investigated the temperature and time of storage, and the effect of the container.

1. Benefits of Breast feeding

- ***Benefits for the child***

The latest Policy Statement from the American Academy of Pediatrics[1] emphasises the benefits of breastfeeding and the use of human milk. Human milk is species-specific, and all substitute feeding preparations differ markedly from it, making human milk uniquely superior for infant feeding. Exclusive breastfeeding is the reference or normative model against which all alternative feeding methods must be measured with regard to growth, health, development, and all other short- and long-term outcomes. There are several thousand studies which validate this statement.

- ***Benefits for the mother***

The mother obtains considerable benefits when breast-feeding and lactating.[2] The benefits include decreased postpartum bleeding and more rapid uterine involution attributable to increased concentrations of oxytocin,[3] decreased menstrual blood loss and increased child spacing attributable to lactational amenorrhea,[4] earlier return to prepregnancy weight,[5] decreased risk of breast cancer,[6-11] decreased risk of ovarian cancer,[12] and possibly decreased risk of hip fractures and osteoporosis in the postmenopausal period.[13-15]

- ***Benefits for the community***

In addition to specific health advantages for infants and mothers, and mothers, economic, family, and environmental benefits have been described in the literature. These benefits include the potential for decreased annual health care costs of $3.6 billion in the United States;[16,17] decreased costs for public health programs such as the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC)[18]; decreased parental employee absenteeism and associated loss of family income; more time for attention to siblings and other family matters as a result of decreased infant illness; decreased environmental burden for disposal of formula cans and bottles; and decreased energy demands for production and transport of artificial feeding products. [19-21] There is little doubt that these savings in the US are reflected in most developed countries.

It is therefore not surprising that the AAP gives an unequivocal recommendation for exclusive breast-feeding when it is not specifically contraindicated (see ICMRA previous reports for details).

However, the AAP also state:

> ***When direct breastfeeding is not possible, expressed human milk should be provided.***

2. Benefits of Feeding Expressed Breast Milk

Much of the published work on the benefits of feeding a child expressed breast milk has involved premature, or low-weight or similarly compromised babies.[22-27]

However there are studies that strongly suggest that expressed breast milk afford the same protection to the child as feeding from the breast. For example studies have shown that infants fed on expressed breast milk have one third less infections than infants fed on a mixture of pasteurised human milk (pasteurisation destroys much of breast milk's protective effect) and formula or on formula alone.[73,84,85] A more recent study strongly suggests that feeding the baby expressed milk rather than substitutes significantly improves cognitive ability (various IQ parameters) as measured at 7 – 8 years old.[83]

In summary therefore there is no doubt that feeding a child with Expressed Breast Milk (EBM) is the next best thing to breast feeding itself.

3. The Storage of Expressed Breast Milk

3.1 Introduction

Surprisingly there seems to be some confusion regarding the best mode for the storage of Expressed Breast Milk (EBM). For example, as recently as 2003 a Q & A session was conducted in the British Medical Journal - - long does expressed breast milk last? Should it be kept in a refrigerator? [28]

One answer pointed out that current guidelines ranged from 24 hours to 8 days in the fridge. [29] It was also stated that EBM could itself delay bacterial growth, keeping it safer for longer than processed milk. However, freezing affects these antibacterial properties. Careful handling during collection and accurate storage temperature was stressed to prevent bacterial contamination and minimise bacterial growth.

Other answers tended to contradictory to this reply in terms of recommended storage times and temperatures.

In the light of this apparent confusion, and also the different standards prevalent for healthy term-babies and for premature babies, an attempt has been made to summarise research papers on the topic of storage and also to incorporate the best advice available on the Internet. This survey ignores the issue of pasteurisation, (57 ^{o}C for 30 minutes).

3.2 Effect of storage time and temperature on the constituents of human milk

It is clear that the longer milk is stored the greater the risk of deterioration of its essential constituents and of an increase in bacterial contamination, even though because human milk is a living tissue, it has numerous immunologic properties that protect it from contamination.[89] Indeed the immunoprotective constituents of human milk are stable when stored at room temperature for 8 h, refrigerated at 0–4°C for 3 days or frozen at -20°C for 12 months.[35]

3.2.2 Deterioration of essential constituents

There is a great deal of information in the literature which describes the changes in human milk on storage.

- **pH**

Perhaps the easiest measure of change is the decline in pH on storage (that is milk because more acidic). Studies have shown that this change amounts to 2 units of pH in when stored for 24 hours at either 15 ^{o}C or 25 ^{o}C. [39,61]

- **Vitamins**

Reduction in Vitamin C content has been of concern to a number of researchers. [31,35] However, these reductions are mostly seen in milk that has been frozen:

Temperature (^{o}C)	Time	Reduction in Vitamin C
-16	1 month	One third
-16	2 months	Two thirds
-20	72 hours	Significant reduction

In addition there has been a report that reduction of Vitamin B_6 can also be found when milk is frozen at -20 ^{o}C. [35]

- **Antioxidants**

The antioxidant Glutathione status (GSH) also shows reduction on storage, even at room temperature for a short period of time: [34,76]

Temperature (^{o}C)	Time	% Reduction in GSH
Room (20-25)	2 hours	73
4	2 hours	79
-20	2 hours	81

- **Free fatty acids**

Storage tends to increase the level of free fatty acids:

Temperature (^{o}C)	Time	Effect
25	Up to 4 hours	Increase in free fatty acids, greater proportion of fatty acids 18:1 and 18:2 released[40]
-20	Several freeze-thaw cycles	Activates lipolysis and increases the production of free fatty acids, monoacylglycerides and diacylglycerides[59]

- **Digestive Enzymes**

In the comparative short term, there appears to be little effect on digestive enzymes, lipase and amylase, test being carried out over 24 hours at temperatures from 15 to 38 ^{o}C. [41,42]

- **Glutathione peroxidase activity**

GPx (glutathione peroxidase activity) significantly decreased in refrigerated and in frozen milk, when compared to their control samples. MDA (malondialdehyde) increased only in refrigerated milk but not in frozen samples. [63]

- **Other effects**

Various effects are summarised in the following table: [40,42-56]

Impact on immunological properties		
	Storage at 0–4^{o}C	**At -20^{o}C**
IgA	NC	NC
sIgA	NC	NC
Lactoferrin	NC	NC
Lysozyme	NC	NC
Fibronectin	NC	
Mucid		
C3 complement	NC	NC
Bifiduum factor	NC	NC
(oligosaccharide)		
a-Tocopherol	Stable	Stable
Cell Count		
Number	NC	Down
Function	NC	Down
Bacterial growth	NC	NC
Inhibition of E.coli	Adherence to Hep-2 cells	NC

NC = No Change

Essentially, freezing inflicts no change not precipitated by previous contamination, the container or exposure to light energy, except for possible lipolysis, demulsification and protein denaturation when thawed. Freezing breaks the emulsion between milk fat globules and the aqueous fraction or the lipid may adhere to the container and is not recovered.

Accordingly, on the basis of this information, one commentator considers breast milk can be safely frozen for 12 mo at -20°C or indefinitely at -70°C with changes only in the cell count and activity and some alteration in the fat globule. [35]

- **Summary of storage effects on milk constituents**

Some of the above changes may be of concern when breast milk is the only food given to premature babies, low weight infants or otherwise compromised children

(for example Vitamin C supplements may be warranted). However, in the main these changes are not significant for healthy babies.

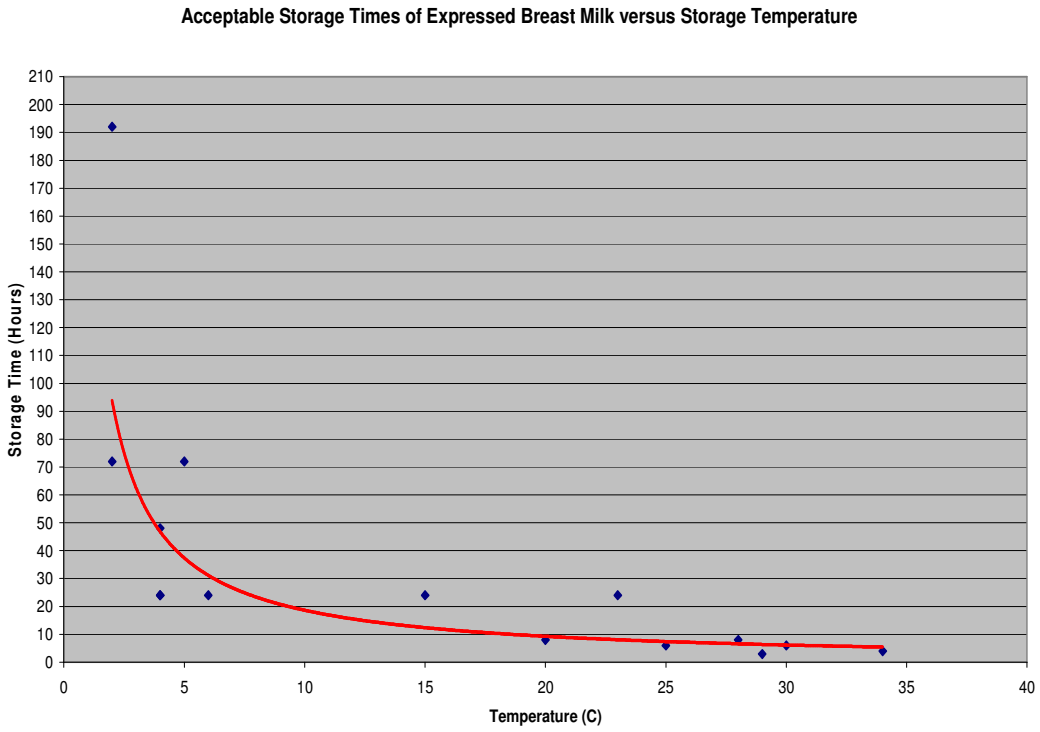
3.2.3 Bacterial Contamination

As well as temperature and time, the degree of bacterial (and mould) growth is a function of the cleanliness of the mother (her breasts and hands) and of the equipment used (see below).

The table seeks to summarise the great deal of research work that has been carried out on bacterial contamination. The table shows the maximum length of time the milk can be stored at the temperature given before bacterial levels reach unacceptability:

Temperature (^{o}C)	Time	Reference
30 - 38	4 hours	32
27 - 32	6 hours	69
29	3 hours	62
28	8 hours	68
15 - 27	8 hours	32
25	6 hours	33
20 - 25	24 hours	77
15	24 hours	33
4 - 10	24 hours	32
4 - 6	72 hours	82
4	24 hours	70
4	24 hours	74
4	48 hours	78
0 - 4	3 days	33
0 - 4	8 days	43
-20	1 month	33
-20	1 month	80

Although this table shows some variation, which is not surprising given the variable milk sample sources etc, a reasonable trend line can be found (omitting storage at -20 ^{o}C):



This shows that at temperatures approaching and below 5 ^{o}C acceptable storage times lengthen considerably and follow a power relationship with temperature.

This is borne out by the advice given to mothers with premature children:

"Breastmilk should be kept at room temperature for as short a time as possible and refrigerated immediately after expression. If a mother is expressing at home and has no access to a fridge, the milk can be kept at room temperature for up to 6 hours. If milk is to be used within 48 hours it should be stored in a refrigerator at a temperature of 2-4°C. Milk which has not been used after 48 hours should be stored frozen (– 20°C) for a maximum of 3 months if it is to be fed to sick preterm infants." [30]

3.2.4 Recommendations for storage times and temperatures

From the above graph a set of guidelines for healthy children may be formulated:

Temperature	Acceptable storage time
Room (20 – 25)	8 hours
Fridge (4)	24 hours
Freezer (-18 or lower)	1 month

It is interesting to evaluate how these calculated figures compare with the advice of researchers and breast milk agencies.

Published recommendations for storage temperatures and times

Temperature						Reference
-20	**0-4**	**5-10**	**15**	**20**	**25**	
	2 days					72
6 months	3 days		24 hours		4 hours	90
6 months	8 days	3 days			6 hours	29
3 months	2 days				3-4 hours	92
3 months	7 days				6 hours	93
3 months	8 days			10 hours		94
6 months	3–5 days		24 hours		4 hours	95
3 months	3 days				6 hours	96
6-12 months	5-7 days		24 hours		4 hours	97
6 months	3 days					98
6-12 months	5 days				6-8hours	99

Therefore the recommended storage times for milk in a fridge (4^{o}C) or a freezer (-20^{o}C) are higher than those calculated above from reported research papers. However this may be because many of the research papers are written with a hospital environment.

When compiling a MAM instruction leaflet it may be wise to err on the cautious side, as follows:

Room temperature	4 hours
Fridge (4^{o}C)	3 days
Freezer (-20^{o}C)	3 months

It is recommended that these storage times and temperatures are used for MAM's instruction leaflets.

Where milk has been stored in a freezer, thawing and refreezing is absolutely <u>not</u> recommended in a number of official leaflets. This is both from a contamination risk point of view but also because there is evidence that such a regime can significantly alter the quality of the milk.

In addition there is a general negative advice for the use of a microwave oven to heat up milk or indeed thaw frozen milk. Reports suggest that this method of heating can result in a possible source of constituent loss - IgA is decreased by 98% and lysozyme by 96%. Microwave radiation of human milk reduces the immunological properties.[52,57]

We would therefore recommend that microwave heating is given as a non-approved method.

3.2.5 Cleanliness

Nearly all of the official recommendations make great emphasis on cleanliness of the mother and of the equipment she is using to both express and store her milk.

A review of the medical literature also serves to emphasis this issue:

All equipment (even breast pumps in a hospital environment) are a potential source of contamination and must be designed to be easily cleaned/sterilised.[86-88]

In some cases chemical cleaning (eg with hypochlorite for the equipment or Phisoderm for breasts) has not proved to be efficient.[60] Washing of breasts with clean water and the use of water at 80 ^{o}C has been shown to be efficacious. Thus MAM should consider materials of construction which can stand the rigours of very hot water, even boiling.

Some researchers have pointed out that significant numbers of EBM samples are grossly contaminated with bacteria, moulds and yeasts. [65-67,75] The sources of this contamination were considered to be the condition of the milk donor's breasts and hands and of the breast pump, where used. Recommendations to reduce the amount of transfers have been made – for example expressing milk directly into a feeding bottle.

3.3 Effect of material of construction of the storage container

The literature (particularly those of a review rather than research nature) abound with advice regarding the material of construction of the storage container. However, in many cases it is difficult to find precise data to confirm or otherwise the recommendations made. The following is a review of the slightly limited amount of data available:

Information on the following materials has been found – glass (mainly Pyrex), steel, polycarbonate, polypropylene, and polyethylene (bags).

- **Glass**

The main advantage of glass is that it can be autoclaved. The material has the disadvantage that it can break (!) chip or crack. There is also a theoretical risk of photo degradation of nutrients in clear glass bottles.[100]

The evidence for the viability of milk cells in glass or indeed plastic bottles (PP or PC) is inconsistent. A study has reported a greater loss of all leucocytes in glass bottles than plastic containers due to cells adhering to the walls[101] which was later confirmed. [45] However other work has not shown a reduction of cells. [37] This may be a function of a difference between ordinary glass and Pyrex or borosilicate glass. For example, Lawrence suggests that in Pyrex, cells adhere to the container walls, whilst with ordinary glass, water-soluble constituents remain stable and is least destructive to the milk. [35]

Two authors are unequivocal regarding the suitability of glass for storage both in the short and long term. [81,86]

- **Steel**

Notwithstanding any drawbacks for this material in the home environment, steel is generally not recommended for storage. Some loss of immunoglobulins have been reported [103] as have changes in cell counts and viability.[58]

- **Polycarbonate**

In the literature the term polycarbonate seems to be interchangeable with polypropylene and therefore it is difficult to provide any real information on this material. One author states that PC should not be recommended for heating milk due to "contamination from plastic residues", but provides no data to prove this contention or indeed makes little comment regarding PC's storage capability. [86]

- **Polypropylene**

The renowned breast feeding authority, Dr Ruth Lawrence considers PP to be ideal for storage as there is no cell adherence, and if rigid, the container can be used for both the freezing and the reheating process. She considers that the water-soluble constituents remain stable. [35] This view is shared by Slusser and Frantz. [86]

However, Goldblum has presented evidence to suggest there is some immunoglobulin loss.[37] Another author has show a fall in Vitamin C as compared with glass, although this did not appear to be significant both statistically or from the welfare of a healthy child point of view. [71] However this latter paper is cited in a number of reviews and is perhaps maybe one source of PP having a slightly negative connotation in hospitals.

It has been stated that PP is particularly suited to the storage of colostrum. [58]

- **Polyethylene (bags)**

The literature has numerous recommendations that polyethylene bags should not be used for EBM storage. [36,64,86,90,91,102]

The main reasons for this advice appears to be that sIgA sticks to the plastic, [37] a loss of nutrient properties (especially fat and fat-soluble vitamins), and the loss of milk or ingress of contamination due to possible leakage/punctures.

In any event, all these recommendations are made in respect of hospitalised children, such as prematures. As far as using PE bags in a normal home environment, Lawrence considers that there is no significant cell adherence [35] and Slusser and Frantz state that this potential leakage due to nicks in the material can be solved by placing the bags in a rigid holder. [86] Indeed the UK organisation – Breastfeeding Network and La Leche league International seem more concerned with this issue of puncturing of the bags than with any other factor.[94,104]

It is clear that much of the controversy surrounding PE bags is that at one time disposable polyethylene bottle liners (such as those marketed by Playtex Inc) were being widely used for storage. [105] Several official organisations are now recommending specially designed bags for milk storage. [92,93,104]

- **Comparison of some materials**

The following summary has been made from papers that allow direct comparisons: [35-38,79]

Effect of container type on milk constituents after 4 h and 24 h storage				
	Pyrex	**PP**	**PE (Bags)**	**Rigid PE**
Colostrum	NC	NC	NC	NC
Volume	NC	NC	NC	NC
Mature milk				
Cell Numbers	Up	Up	NC	NC
Cell Functions	Down	Down	Down	Down
Proteins				
Lactoferrin	Down	Down	NC	NC
Lysozyme	Down	NC	Down	Down
sIgA	NC	NC	NC	NC
Total IgA	NC	NC	NC	NC
Antibodies to E. Coli	NC	NC	Down	Down
Water-soluble vitamin C	NC	Down		
Fat-soluble vitamins	NC	NC	NC	NC

NC – No change

Thus it can be seen that with all reviewed materials some changes take place on storage. However, there is no current suggestion that for healthy children these materials are unsuitable.

4. Recommendations for storage containers

- The system should be simple to use and should keep the number of milk transfers down to a minimum.

- Reusable equipment should be easily cleanable and preferably have the ability to be sterilised by boiling water.

- Materials of construction should be approved for food storage by the U.S. Food and Drug Administration and should conform to the plastics in contact with food regulations in the EU.

- Storage containers should be sold pre-sterilised

- From the above data it would appear that both polypropylene and polyethylene are suitable materials of construction.

5. Some examples of storage systems currently on the market

A brief survey of systems currently on the market has been undertaken.

Avent offer pre-sterilised bases and lids with adaptors to fit their breast pump and feeding nipple.

Bailey Breast Milk Storage Bags are made from polyethylene, which they state helps to "preserve precious nutrients". This of course is at odds with some of the research data already reviewed. They also feature built-in wire closures and write-on end tabs for recording the date and other important information.

Medela's CSF (Collect, Store and Freeze) **Bags** are pre-sterilised for maximum hygiene and they attach directly to any Medela breast shield to allow pumping into the bag. A built-in pouring spout can be torn open when it's time to empty the bag.

Lansinoh Breastmilk Storage Bags have double zipper closures, a colour change seal for proof of closure, a write-on tab outside the fill area with is claimed to eliminate potential punctures and ink contamination.

Gerber Seal 'n Go Breast Milk Storage Bags appear to be similar to the Lansinoh product, certainly in terms of many of the claims made.

Mother's Milk Mate Breast Milk Storage System claims to be a “great” alternative to breast milk storage bags. It is also claimed that the bottles can be attached to most breast pumps with universal seals -- including Medela Pump in Style, Ameda Purely Yours, Whittlestone Breast Expressers, Bailey Nurture III breast pumps (but not the AVENT ISIS). The "first-in, first out" storage rack rotates breast milk storage bottle containers. Critically they claim that all plastic components are made of materials approved for food storage by the U.S. Food and Drug Administration.

A number of other companies offer bottle storage units some with carry cases (for example Ameda).

6. Other considerations

Government documents [102] recommend that mothers should be given written instructions on:

- Guidance on personal hygiene
- Guidance on expressing milk
- Procedure for washing and sterilising all equipment
- Guidance on the labelling, storage and handling of milk

Indeed, a review of recommendations [93-99,104] and of the companies websites mentioned above indicates that this is being undertaken.

Clearly, any leaflet accompanying the final product would have to be carefully drafted.

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