

TITLE: Storage, Handling, and Administration of Expressed Human Breast Milk: A Review of Guidelines

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CONTEXT AND POLICY ISSUES

Expressed human breast milk (EBM) refers to milk that has been expressed from a mother's breast either manually or mechanically using either an electrical or manual breast pump.¹ Human breast milk is generally considered as the optimal source of nutrition for newborns and infants.²⁻⁵ In addition to the nutritional aspect, human breast milk has antioxidant, antibacterial, prebiotic, probiotic, and immune boosting properties.⁶ The benefits of human milk for growth and development of the infant is well established.⁷ It has been consistently demonstrated that for infants, human breast milk is the best nourishment and is beneficial to their health in the short, medium and long term.⁸ In preterm infants, feeding with human milk has been found to reduce infections and necrotizing enterocolitis, to potentially improve neurocognitive and cardiovascular outcomes,⁹ and to reduce hospital readmissions following discharge from the hospital.^{9,10} In Canada, it is estimated that each year there are 25,000 to 30,000 preterm births (gestational age < 37 weeks).¹¹ In Ontario, the preterm birth rate was estimated as 7.9 preterm births for 100 live births for the year 2010.¹²

Although the health benefits of breast feeding are realized, there may be circumstances when the mother may not be able to breast feed. This could be due to breast deformities, hormonal dysfunction, medication use that is incompatible with breast feeding, premature delivery, insufficient milk production, or returning to work,^{2,6,11,13} hence the need for collection and storage of EBM from the mother or donor and its appropriate administration to the infant. There is potential of contamination or loss of nutritional value in EBM if not properly stored and handled. However, there is no agreed-upon definition of unsafe milk.⁶

The purpose of this report is to review the evidence-based guidelines regarding the optimal storage and handling of EBM administered to pre-term infants or healthy infants and young children in the healthcare or community setting.

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RESEARCH QUESTIONS

1. What are the evidence-based guidelines regarding the optimal storage and handling conditions for expressed human breast milk in healthcare settings?
2. What are the evidence-based guidelines regarding the optimal storage and handling conditions for expressed human breast milk in community settings?
3. What are the evidence-based guidelines regarding the optimal conditions for safe administration of expressed human breast milk to pre-term infants?
4. What are the evidence-based guidelines regarding the optimal conditions for safe administration of expressed human breast milk to healthy infants and young children?

KEY FINDINGS

One systematic review, two guidelines, and one report which included amendments to the included guideline were identified. Specific recommendations pertaining to each research question could not be identified. Some general recommendations were available. Furthermore, there was not always agreement in recommendations from the different reports and also not all the same aspects were considered in each of the reports.

According to two guidelines, expressed human breast milk (EBM) should be immediately transferred to the refrigerator or, if possible, immediately frozen. EBM may be safely stored at -20°C for six to 12 months according to one systematic review, up to six months according to one guideline, and up to 3 months according to another guideline. However, according to one systematic review, freezing at any temperature can result in some degradation of immunological properties.

For EBM storage, one guideline recommended containers made of food grade material and one guideline recommended containers made of either glass or BPA-free hard plastic.

METHODS

Literature Search Methods

A limited literature search was conducted on key resources including PubMed, CINAHL, The Cochrane Library, University of York Centre for Reviews and Dissemination (CRD) databases, Canadian and major international health technology agencies, as well as a focused Internet search. Methodological filters were applied to limit the retrieval to health technology assessments, systematic reviews, meta-analyses and guidelines. Where possible, retrieval was limited to the human population. The search was also limited to English language documents published between January 1, 2010 and May 3, 2016.

Rapid Response reports are organized so that the evidence for each research question is presented separately.

Selection Criteria and Methods

One reviewer screened citations and selected studies. In the first level of screening, titles and abstracts were reviewed and potentially relevant articles were retrieved and assessed for inclusion. The final selection of full-text articles was based on the inclusion criteria presented in Table 1.

Table 1: Selection Criteria	
Population	Q1: Infants receiving breast milk in healthcare settings; Q2: Infants receiving breast milk in community settings; Q3: Pre-term (birth at < 37 weeks gestation) infants receiving breastmilk Q4: Healthy infants and young children receiving breastmilk
Intervention	Q1 to 4: Expressed human breast milk
Comparator	No comparator required
Outcomes	Q1 and 2: Guidelines regarding optimal storage (<i>i.e.</i> , storage device, storage temperature, duration of storage, frequency of freeze/refrigerate thaw cycles) Q3 and 4: Guidelines regarding optimal conditions (<i>i.e.</i> , post-storage milk temperature [thaw to refrigerated or room temperature], safe duration post-storage or expression at room temperature) for safe administration of expressed human breast milk to avoid patient harms
Study Designs	Health technology assessments (HTA), systematic reviews (SR), meta-analyses (MA), and evidence-based guidelines

Exclusion Criteria

Articles were excluded if they did not meet the selection criteria outlined in Table 1, they were duplicate publications, or were published prior to 2010. Systematic reviews or guidelines on methods of milk expression or operation of milk banks (such as recruiting and screening donors, training of donors and staff, testing and pasteurizing expressed milk and its transportation) were excluded.

Critical Appraisal of Individual Studies

The included systematic review was critically appraised using the AMSTAR checklist¹⁴ and guidelines were assessed with the AGREE II instrument.¹⁵ Summary scores were not calculated for the included studies; rather, a review of the strengths and limitations of each included study were described narratively.

SUMMARY OF EVIDENCE

Quantity of Research Available

A total of 79 citations were identified in the literature search. Following screening of titles and abstracts, 69 citations were excluded and 10 potentially relevant reports from the electronic search were retrieved for full-text review. One potentially relevant publication was retrieved from the grey literature search. Of these 11 potentially relevant articles, seven publications were excluded for various reasons, while four publications met the inclusion criteria and were

included in this report. These were comprised of one systematic review,¹⁶ two guidelines^{8,17} and one report⁷ which contained amendments to the included guideline.¹⁷ Appendix 1 describes the PRISMA flowchart of the study selection.

Additional references of potential interest are provided in Appendix 7.

Summary of Study Characteristics

One systematic review,¹⁶ two guidelines^{8,17} and one report⁷ which included amendments to the included guideline¹⁷ were identified. Characteristics of the included systematic review and guidelines are summarized below and details are available in Appendix 2 and Appendix 3.

Systematic Review

A description of the included systematic review is summarized below and details are available in Appendix 2, Table A1. The included systematic review was published from Australia in 2015. It included 12 laboratory research studies, published between 2010 and 2014. EBM specimens from mothers were analyzed to determine the effect of storage conditions on nutritional and immunological properties of EBM. The number of mothers providing EBM in the studies ranged between 10 and 90. The systematic review also included three guidelines with recommendations on EBM storage conditions.

Guidelines

A description of the included guidelines is summarized below and details are available in Appendix 2, Table A2 and Appendix 3, Table A3. One guideline¹⁷ was published from Italy in 2010 and some amendments⁷ to this 2010 guideline were published in 2012, by the same group, in accordance with the European Union Commission's Amending Directive. One guideline⁸ was published by the National Institute for Health and Clinical Excellence (NICE) from the United Kingdom (UK) in 2010. Both guidelines focused on the operation of donor milk banks and included guidelines on EBM storage and handling.

Summary of Critical Appraisal

Systematic Review

Critical appraisal of the included systematic review¹⁶ is summarized below and details are available in Appendix 4, Table A4. The objective and inclusion criteria were clearly stated. Exclusion criteria were not explicitly stated. A comprehensive literature search seems to have been undertaken with searching of multiple databases and reference list of relevant articles. The study selection was described but no flow chart was presented. A list of included studies was provided but a list of excluded studies was not. Three reviewers were involved with article selection and data extraction and cross checking. All of the included studies were laboratory research studies. Conflicts of interest of the authors were not mentioned.

Guidelines

Critical appraisals of the two included guidelines^{8,17} are summarized below and details are available in Appendix 4, Table A5. For both guidelines, the scope and purpose were clearly stated and the guideline development groups were comprised of individuals from relevant areas

(such as neonatologists and healthcare workers). One guideline¹⁷ sought input from parents of infants and in one guideline,⁸ the guideline development group included a patient representative. In one guideline¹⁷ recommendations were graded and in one guideline⁸ there was no grading. In one guideline¹⁷ a comprehensive literature search was undertaken but other methodological details were not provided. One guideline⁸ did not specifically describe the methodology but was developed according to NICE guidelines manual which appears to have rigorous methodology. One guideline⁸ was overseen by an independent review panel and for one guideline¹⁷ it was unclear if it was internally or externally reviewed. In one guideline,¹⁷ it was mentioned that the guideline development group had no conflicts of interest and in one guideline⁸ the members of the guideline development group provided their declarations of interest, which were available on the NICE website.

Summary of Findings

Findings from the included systematic review¹⁶ and guidelines^{8,17} are summarized below and details are provided in Appendix 5, Tables A6 and Appendix 6, Table A7. The findings and guidelines were mostly of a general nature rather than being specific to a setting (healthcare setting or community setting) or a particular population (preterm infants, healthy infants or children) as mentioned in the research questions. Hence these general guidelines will be presented first and only guidelines which are specific to each research question, if available, will be presented below the appropriate research question.

Systematic Review

One relevant systematic review,¹⁶ including 12 studies (in the laboratory setting) and three guidelines, was identified. Findings of the individual studies were presented separately. Three studies showed that the nutritional aspects of EBM were maintained when stored refrigerated, however the duration of storage varied among the three studies (four days in two studies, and five days in one study). Two studies showed that the bacteriological aspects of EBM were maintained when stored refrigerated, for two days in one study and for four days in one study. One study found that the immunological properties of EBM appeared to diminish when stored at -20°C for four weeks. Two studies found that the bacteriological aspects of EBM were maintained when stored at -20°C for one month. One study found that the antioxidant properties of EBM were maintained when stored at -20°C for a short duration (not defined). One study found that there was fat loss in EBM stored at -20°C for 30 days, regardless of the type of storage container (a soft polyethylene bag or polypropylene container). Two studies found that freezing affects immunomodulatory proteins in EBM. Two studies showed that prolonged storage (not defined) at -80°C affects the immunomodulatory and anti-oxidant properties of EBM. In addition, some recommendations on EBM storage obtained from three guidelines were presented, however, the evidence on which these recommendations were based, was not described. It was recommended that EBM may be stored at room temperature ($\leq 26^{\circ}\text{C}$) for 6 to 8 hours, in the refrigerator ($< 5^{\circ}\text{C}$) up to 72 hours and in the freezer (-20°C) six months to 12 months. Additional details are available in Appendix 5, Table A6.

Guidelines

Relevant information from the two included guidelines.^{7,8,17} are summarized below and details are provided in Appendix 6, Table A7. The recommendations in one guideline¹⁷ were developed using findings mostly from laboratory studies; a scientific opinion document and a few guidance documents were also considered. The recommendations in the other guideline⁸ were

developed using findings from laboratory studies, narrative reviews, guidance documents and opinion pieces. For EBM storage, the NICE guideline⁸ recommended containers made of food grade material and the Italian guideline^{7,17} recommended containers made of either glass or BPA free hard plastic. The guideline¹⁷ had initially recommended hard plastic containers, and later an amendment⁷ was published, to indicate specifically BPA free hard plastic containers, until further scientific data was available to clarify the toxicological relevance of some effects observed with BPA (such as biochemical changes in the brain and immunomodulatory effects). This guideline¹⁷ also recommended that sterile containers be used for milk collection.

Both guidelines recommended that after collection the EBM should be immediately transferred to the refrigerator or, if possible, immediately frozen. One guideline stated that EBM can be stored in the refrigerator (4°C) up to 72 hours.¹⁷ One guideline¹⁷ recommended that both fresh and pasteurized milk can be stored in the freezer (-20°C) for a maximum of six months, whereas one guideline⁸ recommended that donor milk awaiting pasteurization can be stored in the freezer (-20°C) for a maximum of three months.

One guideline¹⁷ recommended that frozen milk should be thawed slowly in a refrigerator or thawed rapidly in a water bath ($\leq 37^{\circ}\text{C}$) or under running lukewarm water and should not be thawed in a microwave oven. Both guidelines mentioned that frozen milk after being thawed can be kept in the refrigerator for a maximum duration of 24 hours. One guideline¹⁷ mentioned that frozen milk after being thawed should not be refrozen.

One guideline⁸ mentioned that unpasteurized EBM from the same donor can be pooled but batches of pasteurized EBM from the same donor or EBM from different donors should not be pooled.

One guideline⁸ suggested that further research is needed to investigate the comparative effects of milk handling processes on nutritional and microbiological properties and the impact of these on health outcomes of the recipient.

What are the evidence-based guidelines regarding the optimal storage and handling conditions for expressed human breast milk in healthcare settings?

No recommendations specific for the healthcare setting were available.

What are the evidence-based guidelines regarding the optimal storage and handling conditions for expressed human breast milk in community settings?

One guideline¹⁷ recommended that for storage in domestic refrigerators, to minimize risk of contamination, the containers with milk should be stored in a separate container so that they are isolated from other food inside the refrigerator.

What are the evidence-based guidelines regarding the optimal conditions for safe administration of expressed human breast milk to pre-term infants?

One guideline¹⁷ recommended that the maximum period for which EBM may be stored frozen is three months, if the EBM is to be used for feeding preterm neonates.

What are the evidence-based guidelines regarding the optimal conditions for safe administration of expressed human breast milk to healthy infants and young children?

No recommendations specific for the healthy infants and young children were available.

Limitations

Specific guidelines pertaining to each research question could not be identified. Information and guidelines identified were mostly of a general nature rather than being specific to a setting (healthcare setting or community setting) or a particular population (preterm infants, healthy infants or children) as mentioned in the research questions.

None of the studies included in the systematic review were conducted in a hospital, community or home setting, they were research studies conducted in a laboratory setting and reported on nutritional and immunological properties of EBM stored under various conditions. None of these included studies reported on clinical outcomes resulting from changes in nutritional or immunological properties of EBM due to storage. The included systematic review also included recommendations from guidelines, however the evidence on which the recommendations were based was unclear.

There were some inconsistencies in the guidelines and recommendations with respect to storage at -20°C . The reason for these inconsistencies was unclear. The same aspects were not always addressed in the systematic review and guidelines therefore it was unclear if there was agreement between the various reports with respect to aspects such as storage of EBM at 4°C , and procedure for thawing frozen EBM, hence definitive conclusions are not possible.

CONCLUSIONS AND IMPLICATIONS FOR DECISION OR POLICY MAKING

Four relevant reports were identified and were comprised of one systematic review,¹⁶ two guidelines^{8,17} and one report⁷ which included amendments to the included guideline¹⁷. Specific recommendations pertaining to each research question could not be identified. Some general recommendations were available. Furthermore, there was not always agreement in the recommendations from the different reports and also not all the same aspects were considered in each of the reports.

According to two guidelines,^{8,17} EBM should be immediately transferred to the refrigerator or if possible immediately frozen. EBM may be safely stored at -20°C for six to 12 months according to one systematic review,¹⁶ up to six months according to one guideline¹⁷ and up to 3 months according to another guideline.⁸ However, according to one systematic review, freezing at any temperature can result in some degradation of immunological properties.

For EBM storage, one guideline⁸ recommended containers made of food grade material and one guideline¹⁷ recommended containers made of either glass or BPA-free hard plastic. A commentary¹⁸ published in 2014, by the Health Technology Assessment Unit (HTAU) of the Centre Hospitalier Universitaire du Quebec, which did not meet inclusion criteria but had some relevant information. It suggested that plastic specimen containers not be used for storing EBM and other options need to be evaluated for neonatal care units. It commented that the plastic containers used for EBM storage meet most of the characteristics recommended in clinical guidelines, but there is insufficient evidence on its' clinical safety and effects on infants' health. Furthermore, since the container is not marketed for infant feeding, the manufacturer has no legal obligation to ensure it is safe for such use.

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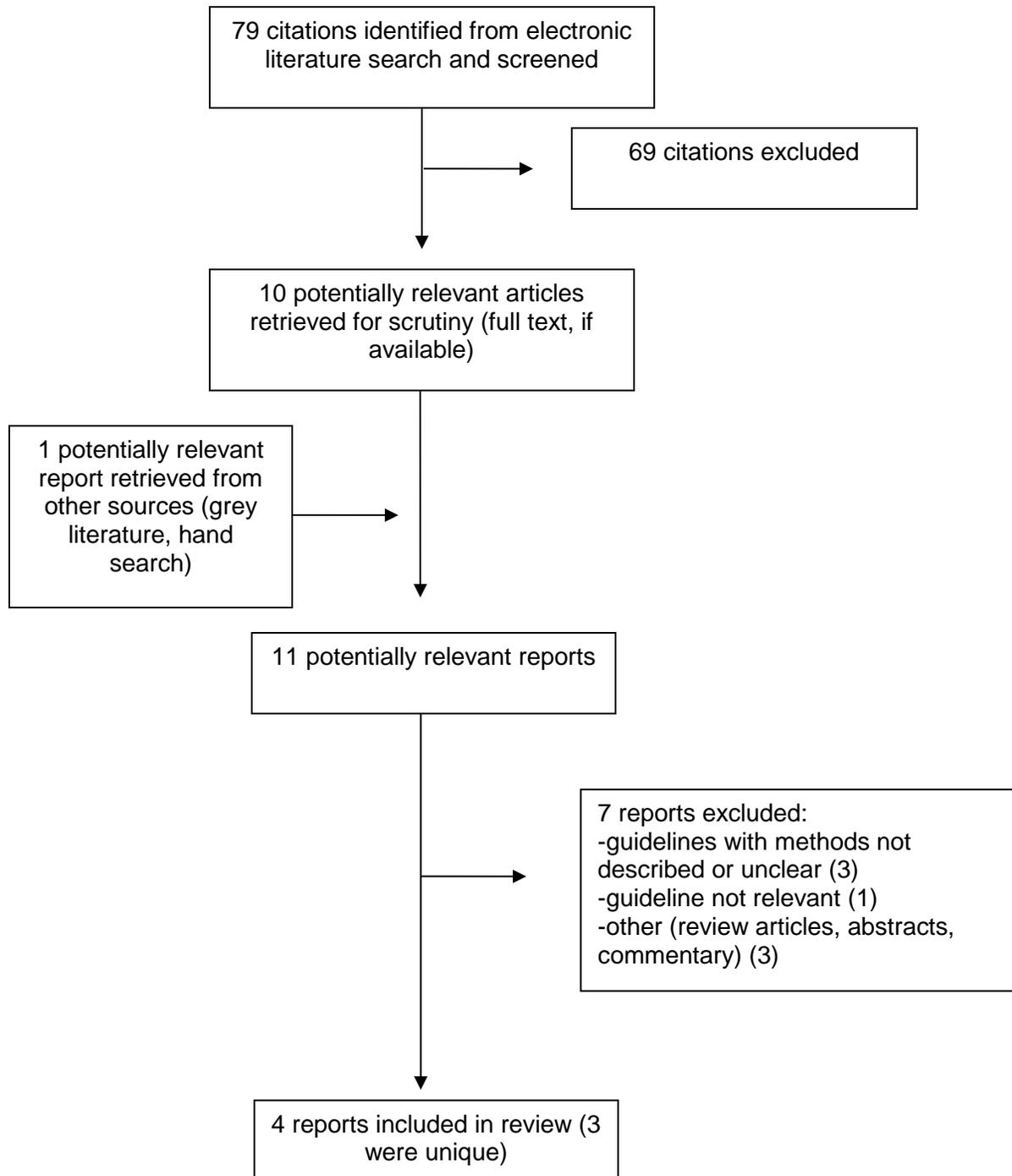
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ABBREVIATIONS

EBM	expressed human breast milk
NICE	National Institute for Health and Clinical Excellence
NICU	neonatal intensive care unit
RCT	randomized controlled trial
SIGN	Scottish Intercollegiate Guidelines Network
UK	United Kingdom

APPENDIX 1: Selection of Included Studies



APPENDIX 2: Characteristics of Included Publications

Table A1: Characteristics of Included Systematic Reviews and Meta-Analyses				
First Author, Publication Year, Country	Types and numbers of primary studies included	Population Characteristics	Interventions	Aspect examined
McArthur, ¹⁶ 2015, Australia	12 experimental studies (bench research) which were relevant for this report. Studies were published between 2010 and 2014 Also three articles (guidelines) from Grey literature were included. Objective: To determine the safe management of EBM in the hospital, home, and community settings.	Mothers providing EBM specimens Number of mothers in the studies ranged between 10 and 90	EBM	Storage conditions

EBM = expressed breast milk

Table A2: Characteristics of Included Guidelines	
Objectives	Methodology
Italian Association of Human Milk Banks,¹⁷ 2010, Italy	
To develop guidelines for optimal functioning of Human Milk Banks in Italy	The guideline development group comprised of a multidisciplinary team: neonatologist, dietician, and nutritionist. Target users: Health care workers of Human Milk Bank, Neonatology department, and NICU, and administrators of hospitals A comprehensive literature search was conducted using multiple database (Medline, Embase [1966 to May 2010] and CINAHL [1981 to May 2010]), and relevant manual search. Levels of evidence and grading of recommendations (based on SIGN) were provided. Discussion between the groups was used for consensus development.
NICE guidelines,⁸ 2010, UK	
To improve the safety of donor milk by making evidence-based recommendations on the operation of donor milk banks.	The guideline development group comprised of a multidisciplinary team: neonatologist, nurse, microbiologist, and patient and care-provider representative. Target users: Donor milk bank staff, healthcare professionals who care for people who use donor milk, and hospitals or organizations considering starting a donor milk bank The guideline was developed according to the NICE guidelines manual (available at www.nice.org.uk/GuidelinesManual) and appears to be rigorous An independent review panel oversaw the development of the guideline. Levels of evidence and grading of recommendations were not provided. Recommendations were developed using formal consensus techniques.
NICE = National Institute of Health and Clinical Excellence, NICU = neonatal intensive care unit, SIGN = Scottish Intercollegiate Guidelines Network	

APPENDIX 3: Grading of Recommendations and Levels of Evidence

Table A3: Grading of Recommendations and Levels of Evidence	
Guideline Society and/or Author, Year, Country, Topic	Recommendation grade and Level of Evidence
Italian Association of Human Milk Banks, ¹⁷ 2010, Italy	<p>Levels of Evidence:</p> <p>“1++ High quality meta-analyses, systematic reviews of RCTs or RCTs with a very low risk of bias</p> <p>1+ Well-conducted meta-analyses, systematic reviews or RCTs with a low risk of bias</p> <p>1- Meta-analyses, systematic reviews, or RCTs with a high risk of bias</p> <p>2++ High-quality systematic reviews of case–control or cohort studies High-quality case–control or cohort studies with a very low risk of confounding or bias and a high probability that the relationship is causal</p> <p>2+ Well-conducted case–control or cohort studies with a low risk of confounding or bias, and a moderate probability that the relationship is causal</p> <p>2- Case–control or cohort studies with a high risk of confounding or bias, and a significant risk that the relationship is not causal</p> <p>3 Non-analytical studies, e.g. case reports, case series</p> <p>4 Expert opinion” Page 4</p> <p>Recommendation Strength:</p> <p>“A At least one meta-analysis, systematic review, or RCT rated as 1++, and directly applicable to the target population, or A body of evidence consisting principally of studies rated as 1+, directly applicable to the target population, and demonstrating overall consistency of results</p> <p>B A body of evidence including studies rated as 2++, directly applicable to the target population, and demonstrating overall consistency of results; or Extrapolated evidence from studies rated as 1++ or 1+</p> <p>C A body of evidence including studies rated as 2+, directly applicable to the target population, and demonstrating overall consistency of the results; or Extrapolated evidence from studies rated as 2++</p> <p>D Evidence level 3 or 4; or Extrapolated evidence from studies rated as 2+ relevant, on which no scientific evidence is available in the literature.” Page 4</p> <p>The following categorization was used in case of recommendations based on studies not directly clinical trials, but assessing qualitative aspects of donor milk.</p> <p>“A* At least two good quality coherent studies, where bias or confounding factors are not present</p> <p>B* At least one study (fulfilling the above mentioned criteria)</p> <p>C* Low quality and/or incoherent studies</p> <p>D* Expert opinion” Page 5</p> <p>Recommendations based on the experience of the members of the guideline development group and considered relevant, on which no scientific evidence is available is indicated by √ (i.e. good practice point)</p>
NICE guidelines, ⁸ 2010, UK	NR
NICE = National Institute of Health and Clinical Excellence, NR = not reported, RCT = randomized controlled trial	

APPENDIX 4: Critical Appraisal of Included Publications

Table A4: Strengths and Limitations of Systematic Reviews and Meta-Analyses using AMSTAR checklist¹⁴

Strengths	Limitations
McArthur ¹⁶ 2015, Australia	
<ul style="list-style-type: none"> • The objective was clearly stated. • The inclusion criteria were stated. • Multiple databases were searched, Also reference list of the relevant articles were manually searched. • Study selection was described but no flow chart was presented • List of included studies were provided • Article selection and data extraction were done by three reviewers and then cross-checked by all three reviewers. • Findings were described narratively 	<ul style="list-style-type: none"> • The exclusion criteria were not explicitly stated. • List of excluded studies were not provided • All the studies were laboratory research studies and hence assigning a level of evidence was not done. • There was no mention of conflict of interest

Table A5: Strengths and Limitations of Guidelines using AGREE II¹⁵

Strengths	Limitations
Italian Association of Human Milk Banks, ¹⁷ 2010, Italy	
<ul style="list-style-type: none"> • The scope and purpose were clearly stated. • The guideline development group comprised of individuals from relevant areas (such as neonatologist, dietician, and nutritionist). • Input was also sought from parents of infants • A comprehensive literature search was undertaken (multiple databases searched, and relevant manual searching). However details of article selection and data extraction were not provided. • Recommendations were graded. • It was stated that guideline development group members had no conflict of interest 	<ul style="list-style-type: none"> • Unclear how article selection and data extraction was conducted. • Unclear if the guideline document was internally or externally reviewed • Cost implications or organizational barriers were not discussed.
NICE guidelines, ⁸ 2010, UK	
<ul style="list-style-type: none"> • The scope and purpose were clearly stated. • The guideline development group comprised of a multidisciplinary team: neonatologist, nurse, microbiologist, and patient and care-provider • The guideline was developed according to the NICE guidelines manual (available at www.nice.org.uk/GuidelinesManual) and appears to be rigorous (search strategy development, article selection, quality assessment, etc.) • NICE guidelines are required to consider both clinical and cost effectiveness. • An independent review panel oversaw the development of the guideline. • Members of the guideline development group 	<ul style="list-style-type: none"> • Unclear if article selection and data extraction were done in duplicate. • Recommendations were not graded.

Table A5: Strengths and Limitations of Guidelines using AGREE II¹⁵

Strengths	Limitations
provided their declarations of interest, which are available on the NICE website	
NICE = National Institute of Health and Clinical Excellence, UK = United Kingdom	

APPENDIX 5: Main Study Findings and Author’s Conclusions

Table A6: Summary of Findings of the Systematic Review

Main Study Findings and Author’s Conclusions

McArthur,^{1b} 2015, Australia

Main Findings:

Twelve relevant articles were identified from the literature search and the findings from each study are summarized below.

Akinbi et al., 2010 (20 mothers): Immunological properties of EBM appear to be diminished when stored frozen at –20°C for four weeks.

Bertino et al., 2013 (17 mothers): EBM stored in the refrigerator for 96 hours seemed to maintain the overall lipid composition.

Hososaka et al., 2013 (20 mothers): Considering safety in terms of bacteriological aspects, EBM was found to be safe for at least two days when stored in the refrigerator (5°C) and for at least one month when stored frozen (–20°C).

Tacki et al., 2013 (22 mothers): In the short-term, bactericidal activity against *Escherichia coli* was found to be better preserved in EBM stored in Pyrex bottles compared to polyethylene bags.

Giribaldi et al., 2013 (17 mothers): EBM stored in the refrigerator for 96 hours seemed to maintain its bioactivity and nutritional quality, without compromising its microbial quality.

Lev et al., 2014 (20 mothers): Storage at –80°C is not recommended as a gold standard practice for prolonged storage of EBM, as nutrient content of the EBM is impacted (significant decrease in energy content [fat and carbohydrate] was found).

Janjindamai et al., 2013 (90 mothers): There is minimal difference between soft polyethylene bags and polypropylene containers, and both types may be used for EBM storage up to 30 days at –20°C. There appears to be significant fat loss in EBM stored frozen at –20°C, regardless of the container type.

Sari et al., 2012 (44 mothers): EBM stored frozen at –80°C for two months did not appear to be optimal for preserving the antioxidant property of EBM.

Rollo et al., 2014 (20 specimens; number of mothers not specified): Refrigerating EBM for five days does not appear to impact nutritional properties (no appreciably decrease in lactoferrin levels). Freezing EBM for three months or more significantly lowers lactoferrin levels.

Silvestre et al., 2010 (10 mothers): For maximal retention of the antioxidant property of EBM, it should be stored frozen at –80°C for no longer than 30 days and for shorter duration when kept frozen at –20°C

Marin et al., 2009 (34 mothers): Storage of EBM at –20°C for six weeks does not significantly affect either the quantitative or the qualitative bacterial composition of EBM.

Chang et al., 2013 (14 mothers): Freezing, heating or pasteurization processes can affect the concentration of immunomodulatory proteins (especially lactoferrin, secretory immunoglobulin A and lysozyme) in EBM.

Three relevant articles providing recommendations were identified from the Grey literature and are presented in the table below..

Recommendations of EBM storage from three articles identified from the Grey literature:

Storage type	Optimal duration of storage
Room temperature (≤ 26°C)	6 to 8 hours
Refrigerator (≤ 5°C)	72 hours maximum
Freezer compartment in refrigerator (–15°C)	2 weeks maximum
Freezer with separate door (–18°C)	3 months maximum
Deep freezer (–20°C)	6 to 12 months maximum

Table A6: Summary of Findings of the Systematic Review

Main Study Findings and Author’s Conclusions

Authors’ Conclusions:

“EBM can be safely stored at $-20\text{ }^{\circ}\text{C}$ for up to 12 months (longest storage reported in literature included in this review was six months in one study), however frozen storage at any temperature will result in degradation of the immunological components of EBM that provide protection against contamination. While outside the capacity of most clinical settings $-80\text{ }^{\circ}\text{C}$ may an option for moderate term (up to two months) storage of EBM (as opposed to long term storage e.g. over two months).” Page 5

APPENDIX 6: Guidelines and Recommendations

Table A7: Summary of Guidelines and Recommendations

Italian Association of Human Milk Banks,^{1/} 2010, Italy

Main recommendations relevant for this report:

Milk storage containers

“It is possible to use both glass and hard plastic containers (A*).

Single use hard plastic containers are preferable to glass containers, as the latter are considered to have a potential risk for operators (wounds due to cutting) as well as for infants (micro particles of glass in milk) (√).” Page 9 (Note: Amendment⁷ to the guideline recommended specifically, BPA free hard plastic containers)

“Soft polyethylene bags are not recommended (B*).” Page 9

Milk storage at home

“The milk collected at home and destined to be donated has to remain at room temperature for the shortest possible time (A*). After each single collection of milk, always in a sterile container, the milk container must be sealed and cooled immediately under running water (A*).”

“Until the transportation of milk to the Bank, two methods can be used for storage: 1. Immediate refrigeration followed by freezing (the method to be used when further additions to the collected milk will be performed) ... 2. Immediate freezing ...

It is not recommended to add freshly expressed milk to frozen milk (√).” Page 9

“The container to be frozen, should never be filled completely. It is recommended, for example, that a bottle of 250 ml should be filled with a maximum of 200 ml of milk (√).” Page 9

“In domestic refrigerators, to minimize the risk of contamination, it is recommended to store the milk bottles in containers that isolate the milk from the other food inside the refrigerator (√).” Page 9

Milk transportation

“The transportation of milk collected at home should be carried out with respect to the “cold chain” in such a way that the milk arrives at the Milk Bank in a frozen state (D).

For a greater safety it is preferable that the Milk Bank itself is responsible for the milk collection at home and its transportation (√).

Transport freezers, thermal bags with dry ice or refrigerating packs can be used.

Avoid the use of common ice (√).” Page 10

Milk storage at the Bank

“Fresh expressed milk collected at the Bank should be transferred immediately to the refrigerator and pasteurized as soon as possible. In exceptional cases, awaiting pasteurization, the milk can be stored in the refrigerator of the Bank at +4°C up to 72 h from the collection (A*). Otherwise it is frozen.” Page 13

“Both fresh and pasteurized milk should be stored in the freezer at a temperature of -20°C.

The maximum duration of storage in a freezer should be 6 months after the date of expression (D).” Page 13

“When human milk is utilized for feeding preterm neonates, the maximum recommended period for storing frozen milk is 3 months (D).” Page 13

“Cooled or thawed pasteurized milk can be stored in the refrigerator at +4°C, and should be used within 24 h (D).” Page 13

Table A7: Summary of Guidelines and Recommendations

Thawing methods

“Frozen milk, either fresh or pasteurized, should be thawed by:

- defrosting the milk slowly in the refrigerator for a max period of 24 h (C), or
- defrosting the milk rapidly in a water bath at a temperature not exceeding 37°C, or under running lukewarm water.” Page 13

“Rapid thawing of the milk should be carried out with particular attention in order to avoid the contact between the cap of the container and the water (D).” Page 13

“The milk expressed and frozen at home, after being thawed, can be stored in the refrigerator for a Maximum period of 24 h before pasteurization.

The pasteurized frozen milk at the Bank, after being thawed, should be used for feeding as soon as possible, or can be kept in the refrigerator for a maximum period of 24 h as mentioned before (A*).” Page 13

“Fresh frozen milk should not be refrozen after being thawed (C*).” Page 13

“Milk should not be thawed in a microwave oven (A*).” Page 13

NICE guidelines,⁸ 2010, UK

Main recommendations relevant for this report:

Storage containers

“Keep all donor milk in containers made of food grade materials.” Page 20

“At all stages, donor milk containers should be labelled clearly for identification.” Page 20

Each container of donor milk should be appropriately labelled with relevant information (such as identification number, storage instruction, and expiry date [no later than 6 months from expression])” Page 21

Home

“.....expressed milk collected for donation should be frozen as soon as possible to maintain the nutritional and microbiological quality of the milk. If this is not possible (for example, because of storage capacity), refrigerate samples collected over 24 hours, and then freeze the batch.” Page 16

“..... expressed milk for donation should remain frozen during storage at home” Page 16

“...if necessary, expressed milk for donation can be stored before transport to the milk bank for up to 3 months in a domestic freezer, at –18°C or lower.” Page 16

Milk bank

“Store pasteurised and unpasteurised donor milk in separate freezers and refrigerators.” Page 18

“Store donor milk awaiting pasteurisation in the freezer at the milk bank (at –20°C) for no longer than 3 months from the date of expression.” Page 18

“Before testing and pasteurising, thoroughly thaw the donor milk, and keep in the refrigerator for no longer than 24 hours. Prevent the donor milk from reaching 8°C while thawing.” Page 18

“Process all donated milk under hygienic conditions (a sterile environment is not necessary). Practise good hand hygiene at all times, and wear gloves whenever handling donor milk.” Page 18

Pooling

Table A7: Summary of Guidelines and Recommendations

“Only pool pre-pasteurised breast milk from the same donor.” Page 18

“Do not pool:
breast milk from different donors,

or

batches of pasteurised breast milk from the same donor.” Page 18

Disposal

“No specific recommendations were made on how donor milk should be disposed of.” Page 101

APPENDIX 7: Additional References of Potential Interest

Guidelines which did not meet the inclusion criteria as methods were not described or unclear

1. Gaikwad M. Breastfeeding: expression of breast milk. Adelaide, Australia: The Joanna Briggs Institute; 2015.
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4. Maternity - breast milk: safe management [Internet]. Sydney, Australia: Ministry of Health, NSW; 2010 Mar 23. [cited 2016 May 20]. (Policy directive). Available from: http://www0.health.nsw.gov.au/policies/pd/2010/pdf/PD2010_019.pdf
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6. Breast milk: expressing and handling [Internet]. London: Great Ormond Street Hospital for Children; 2014. [cited 2016 May 20]. (Clinical guidelines). Available from: <http://www.gosh.nhs.uk/health-professionals/clinical-guidelines/breast-milk-expressing-and-handling>
7. Breastfeeding: pumping and breastmilk storage [Internet]. Washington (DC): U.S. Department of Health and Human Services, Office on Women's Health; 2015 Aug 3. [cited 2016 May 20]. Available from: <http://www.womenshealth.gov/breastfeeding/pumping-and-breastmilk-storage.html>
8. Ref ID: 33
Expressed breast milk - freezing, storage and checking of [Internet]. New Lambton Heights, Australia: Hunter New England Local Health District; 1995. [cited 2016 May 20; revised 2011 Sep]. (Guideline). Available from: http://www.kaleidoscope.org.au/site/content.cfm?page_id=356024¤t_category_code=8337&lca=930
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