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Evidence Check

Safe management of Expressed Breast Milk (EBM)

An **Evidence Check** rapid review brokered by the Sax Institute for NSW Kids and Families. December 2014.

NSW KIDS + FAMILIES

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This report was prepared by:

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June 2015

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Suggested Citation:

McArthur A, Peters MDJ, Munn Z, Chu WH. Safe management of Expressed Breast Milk (EBM): An Evidence Check review brokered by the Sax Institute (www.saxinstitute.org.au) for NSW Kids and Families, December 2014.

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Safe management of Expressed Breast Milk (EBM): a rapid review

An **Evidence Check** rapid review brokered by the Sax Institute for NSW Kids and Families, December 2014.

This report was prepared by Alexa McArthur, Micah D J Peters, Zachary Munn, Wing Hong Chu

NSW KIDS + FAMILIES

Contents

1 Executive summary

This evidence check review presents a summary of the evidence around how to safely manage expressed breast milk (EBM) in hospital, home and community settings. Four main issues are the focus of this review.

- EBM may become contaminated by viruses and bacteria due to factors including blood-borne maternal transmission and improper handling.
- EBM may lose its nutritional value or become contaminated due to improper storage or transport.
- EBM may become contaminated due to improper disinfection and/or cleaning practices during expression and/or storage.
- There is always a small risk that a baby ('wrong baby' as opposed to the 'right baby') unintentionally receives EBM that does not come from the baby's mother.

The literature search was conducted in a hierarchical manner with evidence first sought from academic papers published from 2008 onwards in Medline (via PubMed), CINAHL, EMBASE, and the JBI Database of Systematic Reviews and Implementation Reports.

The literature search located 7093 articles for all questions combined. After screening, selection at full text level and examination of the reference lists of included papers 43 articles remained for quality appraisal and inclusion in the review. In general, the quality of included studies ranged from low level evidence bench research studies to high quality systematic reviews.

Regarding risks of pathogen transmission from mother to child, benefits for the infant to continue receiving EBM must be considered against the possible severity of the disease. Some pathogens (e.g. HIV) with clear evidence of transmission through breast milk can cause significant morbidity and mortality, and in these cases the administration of breast milk should be avoided. The potential risk for transmission of pathogens through EBM misdelivery, although extremely low, is considered a hospital error and poses a risk to patient safety.

In regard to the impact of storage of EBM, EBM can be safely stored at –20 °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 °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).

The type of container in which EBM is stored seems to have a very slight impact upon bactericidal activity and nutrition of EBM.

The bacteriological profile of EBM at the time of expression seems to directly influence the composition of EBM tested after storage. Adequate cleaning and disinfection of EBM expression and storage equipment should be performed in all contexts. This is especially necessary for shared equipment.

Existing grey literature guidelines and recommendations from Australia, the UK, and the USA provide clear guidance around advisable storage temperatures, durations, and disinfection/cleaning of EBM equipment however, they do not discuss the risks of contamination or impact upon nutritional content.

Regarding the risk of misdelivery of EBM, continuous education, training and monitoring of the policy is recommended. Although it may not be feasible at this point in time, bar coding processes and the establishment of dedicated milk technicians may be considered and discussed in the future.

The results of this evidence check review uphold many of the findings and recommendations of the current policy developed by NSW Health (Maternity – Breast Milk: Safe Management). Regarding the storage and transport of EBM as well as the handling, cleaning and disinfection practices surrounding its collection and storage, the latest NHMRC infant feeding guidelines are accurate and in concurrence with the evidence located in this review.

In individual units where misdelivery of EBM is occurring, healthcare professionals may like to consider implementing quality improvement initiatives and risk analysis to implement strategies (potentially in line with the NSW Health policy) to reduce this risk.

2 Introduction

This paper presents a review of evidence around how to safely manage expressed breast milk (EBM) in hospital, home and community settings. Four main issues are the focus of this review.

- EBM may become contaminated by viruses and bacteria due to reasons including blood-borne maternal transmission and improper handling.
- EBM may lose its nutritional value or become contaminated due to improper storage or transport.
- EBM may become contaminated due to improper disinfection and/or cleaning practices during expression and/or storage.
- There is always a small risk that a baby ('wrong baby' as opposed to the 'right baby') unintentionally receives EBM that does not come from the baby's mother.

While some of the literature on transmission may apply to both breastfeeding and EBM feeding modes, the focus of the review is on EBM. Literature on milk banking is also excluded from the review.

Background

Breast milk is the ideal form of nutrition for all newborns, even those who are born pre-term or are ill.[1] However, it may be difficult or impossible to breastfeed some newborns, particularly those who require care in the neonatal intensive care unit. When breastfeeding is not feasible, breast milk can be expressed and delivered via another method.

Risks of pathogen transmission from mother to child

Maternal pathogen transmission via breast milk may pose a risk for postnatal maternal-to-child transmission of viruses and bacteria. Once these pathogens have entered EBM they may be delivered either to the right or wrong baby, resulting in varying outcomes. The pathogen may not be present in EBM, or may be present and transmitted posing a risk to the infant.[2] Human milk contains antibodies and other protective factors, but in the case of certain pathogens, it may also pose a possible risk of infection for the infant. The benefits for the infant to continue receiving EBM must be considered against the possible severity of the disease. There are few pathogens with clear evidence of transmission through breast milk that cause significant morbidity and mortality, but in these cases the administration of breast milk should be avoided.[3]

Breast milk misdelivery, when a baby receives breast milk from someone other than their own mother, occurs at a rate of 0.07 – 0.14 per 1000 NICU feedings.[4] All people involved in this error (the baby, his/her mother and the woman whose breast milk was inadvertently administered) are tested for pathogens that may be transmitted via breast milk. In most circumstances, the results of the testing are negative, and no harm is caused to the baby. However the importance of misdelivery of EBM cannot be underestimated, and it is considered as a hospital error which poses a threat to patient safety. [4]

Storage and transport of EBM

Mothers may need – or choose – to feed their infants EBM. Mothers may collect EBM at home and transport it to work or to a daycare centre for their infant to consume later.[5-7] A recent systematic review has shown how the incidence of milk expression among mothers has increased in conjunction with the availability of infant feeding equipment.[8]

In the hospital context it may be necessary to store EBM onsite or transport EBM between sites to feed infants. In all of these cases it is necessary to know how to safely store and transport EBM in order to best preserve its nutritional content and reduce the likelihood of contamination and potential infection.[9] Mothers and hospitals may refrigerate or freeze EBM in the short term (e.g. several hours or days) or for longer periods (several weeks to months). Differences in the duration and temperature of EBM storage have been examined and have been found to have a number of impacts upon the quality of EBM in terms of bacterial contamination and nutritional profiles.

Impact on contamination

Human milk is not a sterile substance and even when expressed after careful personal hygiene, EBM will naturally contain a host of non-pathogenic regular bacteria and skin flora at levels that are, in normal circumstances, safe for infant consumption.[10] A number of these normal bacteria are even healthy for infants and contribute to the development of the microbacterial environment in the infant digestive system.[11] Lower levels of bacteria at the start of storage typically result in reduced bacterial growth over the storage period.[11]

Storage of EBM may afford these normally present and harmless bacteria the opportunity to proliferate above typical levels and may also provide opportunities for other non-native bacteria to contaminate the EBM from other sources related to expression, storage and transport.[11]

Impact on nutrition

Human milk is often cited to be the optimum food for infants as it contains a complete range of nutrients necessary for normal, healthy development and growth. Storage of EBM for any amount of time and temperature may impact upon the concentrations of these nutrients. In many studies, freezing has been found to denature some nutritional components in EBM over time. This means that while frozen storage may be useful for preserving EBM for long term use, its nutritional quality can be compromised.

Impact upon immunological components of EBM

Additional to microbial and nutritional content, human milk also contains an assortment of immunological components. These components of human milk are both beneficial for infants – especially pre-term infants – and also offer bactericidal protection to the EBM itself. As with the nutritional quality of EBM, these immunological components have been found to be adversely impacted upon by storage, especially at very low temperatures for prolonged periods of time.

Expressing EBM: disinfection and cleaning equipment, hands and breasts

As with storage and transport, microorganisms can multiply when the milk and the containers used to collect and store it are not disinfected or cleaned properly or if the method of milk expression –such as using a breast pump or manually using the hands – is not done hygienically. Recommendations have been developed for good hygienic practices for the expression, collection, and optimal handling of EBM.[11,12] It is also necessary to follow some hygienic practices when cleaning and disinfecting breast pumps, containers, hands, and the breasts in order to ensure that EBM is not contaminated at the time of expression and collection.

Misdelivery of EBM

In any clinical area where EBM is stored prior to being delivered, there is a small but significant risk for error to occur and for milk to be delivered to the wrong infant. In cases where the breast milk is contaminated, this is a particularly serious error. However, even if the milk is not contaminated, unwittingly providing another mother's milk to one's own infant can have a potentially distressing impact on the mother and result in mistrust of the healthcare provider and significant costs.[13]

Review questions

Four review questions guide this review of evidence:

1. What are the risks of pathogen transmission from mother to child?
2. What is the impact of storage and transport of EBM on contamination and nutritional quality of milk?
3. What is the evidence about disinfection and cleaning procedures for handling EBM?
4. What is the evidence about operational procedures that can minimise the risk of EBM misdelivery?

3 Methods

Literature search

This review first sought peer-reviewed articles published from 2008 onwards. The literature search was conducted in a hierarchical manner with evidence first sought from academic papers. As a sufficient amount of evidence of adequate quality was located for each question, it was not necessary to examine grey literature.

Evidence was first sought from countries with similar income to Australia, such as the USA, the UK and selected OECD countries, as in this context, similarity of resources was deemed more important than similarity of health care systems.

The first phase involved a search of citation databases; including Medline (via PubMed), CINAHL, EMBASE, and the JBI Database of Systematic Reviews and Implementation Reports. The search terms used were: breast milk, human milk, and express*.

The subject headings and indexing terms for each citation database were also added to the key search terms to maximise the capture of the literature on expressed breast milk.

The key search terms were combined and tailored for each question and each database. See Appendix 1 for a detailed report of the search strategy.

Selection criteria for this review were developed according to the scope for each question (see Appendix 2).

The literature search located 7093 articles for all questions combined. The titles and abstracts of 2830 located articles were first screened after 4263 duplicates had been removed and 109 potentially relevant sources of evidence were retrieved in full text for more detailed review. Ten articles were located from the reference lists of included articles. After full text review 43 articles remained for quality appraisal.

Quality appraisal

The full text of the 43 selected articles were assessed and appraised independently by three reviewers using predetermined criteria for determining the quality of the evidence (see Appendix 3). The reviewers also assigned each article a level of evidence according to the NHMRC Levels of Evidence hierarchy (see Appendix 4).

- Question 1: the quality of the included articles varied, with two high quality systematic reviews through to 'low' or 'very low' quality (including bench research) and expert opinion based literature reviews.
- Question 2: Due to the nature of the research required to establish the impact of storage and transport on contamination and nutritional quality of EBM, the articles for this question were exclusively bench research. Bench is counted as 'Level 5c' in the Joanna Briggs Institute's Levels of Evidence.[14] The NHMRC does not have a level of evidence for bench research.
- Question 3: The quality of the articles varied, with one high quality systematic review as well as a low quality case series and expert option based literature reviews.
- Question 4: All articles were either 'low' or 'very low' quality. Only one article followed the traditional reporting structure of providing an introduction, methods, results and discussion.[15]

Data extraction

Three reviewers extracted relevant data to answer the four review questions from each article into tables (Appendix 5). This was cross-checked for completeness by all three reviewers.

4 Results and analysis

The results and analysis are presented by review question below. Identified gaps in the evidence base have been reported specific to each research question.

Review Question 1: What are the risks of pathogen transmission from mother to child?

Twenty papers were identified that met the selection criteria for Question 1 (see table 1).

Table 1. Foci of included studies in relation to Question 1

Study	Bacteria	HIV	CMV	HBV	HCV	HTLV I & II	HSV I & II	Rubella	Syphilis	VZV
Civardi et al. 2013.2	√								√	
Widger et al. 2010.16	√									
Davanzo et al. 2013.17	√									
Landers et al. 2010.18	√									
Schanler et al. 2011.19	√									
Serra et al. 2013.20	√									
Horvath et al. 2009.21		√								
White et al. 2014.22		√								
Henrick et al. 2012.23		√								
Hoque et al. 2013.24		√								
Ehlinger et al. 2011.25			√							
Hayashi et al. 2011.26			√							
Nijman et al. 2012.27			√							
Capretti et al. 2009.28			√							

Shi et al. 2011.29				√						
Pfaender et al. 2013.30					√					
Cottrell et al. 2013.31					√					
Takeuchi et al. 2010.32						√				
Matsubara et al. 2014.33						√				
Hayakawa et al. 2010.34								√		

Bacteria

Bacteria commonly isolated from breast milk, including *staphylococci*, *streptococci*, and lactic acid bacteria, should be considered components of the natural microbiota of breast milk rather than bacterial contamination. Human milk is an important factor in the initiation, development, and/or composition of the infant gut microflora, therefore, the non-sterility of breast milk can be seen as a protective factor. Some of the bacterial strains transferred to the infant gut through breastfeeding have a high probiotic potential and may contribute to the protective effect of breast milk against infectious diseases.[2]

In most cases the potential benefits of breast milk outweigh the possible negative effects of contamination and breastfeeding is not contraindicated. Evidence from mostly low-level case studies[16,19] reported that random milk cultures obtained early in the postpartum period or at any stage, are not predictive of infections in premature infants. One study reports on the process of Holder pasteurization as an effective means to remove any detectable bacteria from samples of donor human milk.[18] In clinical practice, culture of breast milk is generally not practical or necessary.

Human Immunodeficiency Virus type 1 (HIV)

Mother-to-child transmission (MTCT) of human immunodeficiency virus type 1 (HIV) is the most common means by which children acquire HIV infection, and may occur during pregnancy, labour or breastfeeding. There are several risk factors for transmission of HIV from mother to child. Maternal viral load (the amount of HIV RNA in the plasma) is identified as the strongest independent determinant of the risk of MTCT.[21] Complete avoidance of breastfeeding is efficacious in preventing MTCT of HIV. Where affordable, feasible, acceptable, sustainable, and safe alternatives to breast milk are available, it is recommended that HIV-infected mothers do not feed their infant with their breast milk.[22] Many of the studies assessed for inclusion reported on HIV-infected women breastfeeding their infants in resource-limited settings, so lie outside the scope of this evidence check.[35] Heat-treated milk appears to have similar nutritional and immune composition as untreated milk, however this needs further research.[24] The World Health Organization (WHO) also endorses the need for further research to find safe, feasible, and effective methods to heat-treat expressed breast milk.[24,36]

In regards to delivery of EBM to the right baby, or misdelivery to the wrong baby, heat-treatment may provide future safe alternatives to current clinical practice, but at this stage cannot be recommended in clinical practice within the Australian setting.

Soluble Toll-like receptor 2 (sTLR2) in breast milk may be an important factor in infant health and be beneficial in decreasing vertical HIV transmission to infants.[23] Further research into the protective role of EBM would be of assistance in ascertaining the pathogen risk for EBM delivery from the HIV-infected mother to her baby. In the case of misdelivery of EBM to the wrong baby, the amount of any protective element would simply not be known.

Cytomegalovirus (CMV)

Human cytomegalovirus is the most common viral infection in pregnancy, with an estimated prevalence of 0.6 – 2.2%. Congenital CMV is the leading cause of hearing loss in children.

Vertical transmission from mother to fetus can occur in utero, during birth, or from receiving breast milk. The only route of transmission that has been associated with permanent disabilities is in utero.[3] Evidence from four included studies of moderate to high quality suggest that CMV infection via fresh breast milk is mild, self-limiting, and without sequelae. There is an association with infection in very premature infants with pre-existing chronic disease. The possibility of using frozen-thawed or pasteurised breast milk is mentioned in these studies but the impact on the nutritional and immunological elements of breast milk remain unclear.[26,28]

In regards to delivery of EBM to the right baby, or misdelivery to the wrong baby, heat-treatment or freezing may provide future safe alternatives to current clinical practice, but at this stage cannot be recommended in clinical practice within the Australian setting.

In regards to delivery of CMV-infected EBM to the right baby, evidence suggests that virologic or immunologic factors in milk may be associated with decreased risk and severity of postnatal CMV infection. With delivery of CMV-infected EBM to the wrong baby, the difference in risk is simply not known.

Hepatitis B (HBV)

Hepatitis B infection is gradually being reduced as the widespread campaign to vaccinate infants increases immunisation rates. Current Australian recommendations are that all infants are vaccinated against hepatitis B within 24 hours of birth.[37] The birth dose of hepatitis B vaccine has been reported to be well tolerated by newborn infants. In hepatitis B-positive mothers, breastfeeding may commence as soon as the infant has been immunised.[12]

Evidence from a systematic review suggested that exposure to breast milk after immunoprophylaxis did not contribute to mother-to-child transmission of HBV and is recommended.[29] This is irrespective of whether the breast milk has been delivered to the right or wrong baby.

Hepatitis C (HCV)

Hepatitis C virus RNA has been detected in breast milk, but the possibility of transmission is thought to be extremely low. The risk of transmission is higher if the mother has cracked or bleeding nipples. There is no recommendation against breastfeeding from HCV-infected women either from the American College of Obstetricians and Gynecologists (ACOG) or the European Association for the Study of the Liver (EASL).[30] Women who are both Hepatitis C and HIV positive should be managed by the HIV clinical protocol.[12]

Evidence suggests that breast milk does not increase the risk of transmission of HCV, and may even be protective due to immunological benefits. HCV RNA has been detected in breast milk, but with an extremely low viral count, and is thought to be inactivated by the gastric pH of the infant's digestive tract.[38]

Human T cell Leukaemia virus type I & II (HTLV I & II)

HTLV-I can be transmitted in breast milk; breastfeeding is the most prevalent, but not the only route of mother-to-child transmission, and the longer the duration of breastfeeding, the higher the risk of MTCT. The virus occurs in general populations in Japan, the West Indies, parts of Africa and South America, and in many indigenous populations in central and northern Australia.[33,39] Avoidance of breastfeeding is recommended.

HTLV-II antibodies have been detected in breast milk, and have also been isolated from individuals other than leukaemia patients. The epidemiology of transmission to the baby and risk of subsequent disease are unclear. HLTV-II has been identified in some indigenous populations and the risk of transmission is considered to be extremely low.[33]

Herpes simplex virus types I & II (HSV I & II)

HSV infection of the fetus or newborn may be caused by either HSV type I or type II virus, although it is thought that HSV-1 may be more readily transmitted to the neonate.[40] Most neonatal infections (85%) result from exposure to HSV during delivery, and the risk of MTCT is greater among women who acquire a primary HSV genital infection (25 –60%) near the time of birth, than those who experience a reactivation of the HSV infection (1 –5%).[3] Postnatal transmission is relatively uncommon and may account for 10% of cases. Direct contact between lesions on the mother's breasts and the infant's mouth should be avoided until all active lesions have resolved.[12]

No studies were identified that reported specifically regarding transmission of HSV I & II via breast milk. Low level evidence from literature reviews was identified that addressed the epidemiology, diagnosis, and prevention of MTCT for HSV infection only.

Rubella

Rubella virus is a pathogen which can result in severe birth defects, known as congenital rubella syndrome (CRS), if the infection occurs during pregnancy. Since the introduction of vaccination programs, rubella and CRS have been drastically decreased in developed countries.[34] Low level evidence suggested that breast milk has a possible benefit in relation to protecting against the transmission of the rubella virus infection.

Syphilis

There is no evidence that syphilis can be transmitted via breast milk. If syphilitic lesions involve the breast or nipples, then breastfeeding or using expressed breast milk should be delayed until the mother has completed pharmacotherapy and the local lesions have healed. Maternal syphilis infection may justify temporary avoidance of breastfeeding.[2,12] Syphilis infection in the mother, with clinical features of syphilitic lesions on the breast has been associated with the transmission of the virus.

No studies were identified that reported specifically regarding transmission of syphilis via breast milk. Low level evidence from a literature review, and evidence-based guidelines were identified only.

Varicella Zoster Virus (VZV)

Varicella zoster virus is a highly contagious and notifiable disease. Breast milk is not considered to be a significant route of transmission for VZV, and with infants exposed through maternal varicella zoster, breastfeeding continues to be encouraged and the infant may be given newborn zoster immunoglobulin. [37]

No studies were identified that reported specifically regarding transmission of varicella zoster virus via breast milk.

Gap analysis

Overall in the section reporting on the risks of pathogen transmission from mother to child via EBM, the level of evidence is low and there are many gaps in the research literature. We were unable to locate

primary studies which provided high quality evidence addressing the risks associated with misdelivery. The difference in risk between delivery to the right or the wrong baby is simply not known, apart from acknowledging the significant immunological benefits that breast milk provides when delivered from the baby's own mother.

Much of the research literature addressed issues of relevance to breastfeeding over a period of time, and not a one off accidental exposure of EBM being delivered to the wrong baby. Within the HIV research literature, many studies are focused in less resourced countries, and are therefore outside the scope of this evidence check.[36] Techniques such as heat treatment of EBM has been reported to be a simple and cost-effective approach to decrease vertical HIV transmission in less resourced contexts,[24] but further research is required as to whether this could provide possible benefits within other settings.

Review Question 2: What is the impact of storage and transport of EBM on contamination and nutritional quality of milk?

Twelve papers were identified that met the selection criteria for Question 2 (see table 2), all of which reported on the impact of storage of EBM upon contamination, nutritional quality and the immunological components of EBM. Papers also reported on the impact of container type on EBM.

Table 2. Foci of included studies in relation to Question 2

Study	Storage Duration	Transport	Contamination	Immunology	Nutrition	Temp (degrees °C)	Container Type
Takci et al. 2013[41]	24 and 48 hours		√			4	√
Lev et al. 2014[42]	1 to 10 weeks				√	–80	
Janjindamai et al. 2013[43]	4 weeks		√		√	–20	√
Sari et al. 2012[44]	2 months				√	–80	
Rollo et al. 2014[45]	3 and 6 months			√		–18, –20 and 4	
Silvestre et al. 2010[46]	15, 30 and 60 days				√	–20	
Akinbi et al. 2010[47]	4 weeks			√		–20	
Bertino et al. 2013[48]	96 hours				√	6.8 (mean)	
Hososaka et al. 2013[49]	3 hours, 6 hours, 48 hours and 4 weeks		√			20, 5 and –20	
Giribaldi et al. 2013[50]	96 hours		√		√	6.8 (mean)	
Marin et al.	6 weeks		√			–20	

2009[51]							
Chang et al. 2013[52]	4 weeks			√		–20	

Storage and temperature control

All twelve included studies reported upon the storage of EBM. Two studies specifically reported on aspects of temperature control for the storage of EBM. The authors of these two studies continuously monitored the temperature of the refrigerator used in their experiment and noted that despite setting the thermostat to 5 °C, the mean temperature throughout the study was 6.8 °C (range between 4 and 9.6 °C).[48,50] This indicates that temperature measurement at a given moment may not represent the true operating conditions of the refrigeration device used to store EBM. This is an implication that may be relevant for the storage of EBM by health workers and families using domestic refrigerators.

Bacterial Contamination

Four papers examined issues around the impact of storage on the contamination EBM.[41,49-51]

When stored at a mean temperature of 6.8 °C for 96 hours, overall bacterial composition of EBM remained unaffected from the time of collection, indicating that EBM can be stored for up to 96 hours without compromising microbial quality.[50]

The bacteriological profile of EBM at the time of expression seemed to directly influence the bacterial count after storage regardless of method or duration. This indicates that care to avoid contamination must be taken at the time of expression.[49] Expressed breast milk stored for four weeks at –20 °C remained safe for consumption in terms of bacterial content.[49] Bacterial counts declined significantly due to frozen storage. While the immunological components of EBM were not measured, the authors speculated that the rates of bacterial count were related to the activity of immune factors in human milk based on the observation that bacterial counts in refrigerated EBM at 48 hours were lower than for fresh, frozen and room temperature EBM.[49]

In another study, frozen storage of EBM at –20 °C for six weeks did not significantly affect the bacterial composition of EBM.[51] As with the previous study, bacterial content of the EBM at the time of collection was related to content at the end of the study. These results support the safe storage of EBM at –20 °C for up to six weeks.

Takci et al. evaluated the impact of container type (Pyrex bottles versus polyethylene bags) for the maintenance of the bactericidal activity of EBM at 24 and 48 hours (4 °C) against *E coli* contamination.[41] The results indicated that Pyrex bottles are preferable to polyethylene bags for the short term storage of EBM.[41] Another study by Janjindamai and colleagues assessed differences between hard polypropylene (HC) and soft polyethylene bags (SB) in terms of bacterial contamination.[43] Only non-pathogenic organisms were detected in all but one sample from one mother. No significant differences between container types were noted in terms of bacterial contamination outcomes at –20 °C.

Immunological components of EBM

Three papers discussed the impact of storage on the immunological components of EBM.[45,47,52]

Frozen storage of EBM for four weeks at –20 °C reduced the activity of immunological components (lysozomes, immunoglobulin A, lactroperoxidase, muramidase, lactoferrin, and peroxidase) of EBM.[47,52] Reduction in the activity of these EBM components may be associated with an increase in the capability of bacterial proliferation which may have clinical relevance for the use of frozen EBM in terms of its resistance to bacterial contamination.[47] Rollo and colleagues investigated the impact of –18 °C and –20 °C storage (for

three and six months) on the lactoferrin concentration of EBM.[45] The greatest losses of lactoferrin occurred due to the initial activity of freezing, however decreases continue throughout six months of storage. Lactoferrin concentrations in EBM stored for five days at between 4 °C and 8 °C were stable as compared to freshly expressed EBM.[45] The results of these studies indicate that any low temperature storage has a deleterious impact upon the immunological functions of EBM. Frozen EBM may therefore have a compromised ability to defend against microbial contamination and may also not be optimal for infants' uptake of immunological components from fed milk.

Nutritional quality of EBM

Six papers discussed the impact of storage on the nutritional quality of EBM.[42-44,46,48,50]

Refrigerated storage of EBM for up to 96 hours at a mean temperature of 6.8 °C did not seem to adversely impact upon the lipid/fatty acid composition and oxidative status of EBM.[48] In a study with storage conditions that were identical to the previous study, probiotic lactic acid bacteria were not affected adversely by storage.[50] These results uphold the previous findings cited in the 2012 NHMRC Infant Feeding Guidelines that fresh EBM may be safely stored in refrigerator conditions for up to 96 hours.[12] Silvestre et al. examined the impact of frozen storage (–20 °C and –80 °C) on glutathione peroxidase activity and malondialdehyde concentration in EBM.[46] This study reported that any frozen storage of EBM results in the loss of antioxidant properties, but authors observed that –80 °C storage resulted in less negative impacts upon antioxidant activity, especially at the beginning of storage (less than 30 days). Summarily, the authors recommend that for short–term storage of EBM (maximum 30 days), –80 °C should be recommended over –20 °C.[46]

At –20 °C stored in hard polypropylene containers (HC) and soft polyethylene bags (SB) for four weeks, no significant difference in the fat content of EBM was observed between samples apart from very slight losses in the HC containers potentially due to adhesion of fat molecules to the container walls.[43] The authors did note however that significant fat loss was observed for both container types, indicating that storage of EBM at –20 °C for four weeks was the main factor behind fat loss rather than the type of container used.

Lev and colleagues assessed the impact upon deep-freezing EBM at –80 °C for between one and 10 weeks (eight to 83 days) in terms of fat, carbohydrate and energy content.[42] The study found that energy content from fat and carbohydrates was significantly reduced in stored EBM. This decrease was also significantly higher than losses previously reported at –20 °C. Summarily, the authors do not recommend EBM should be subjected to long-term storage at –80 °C.[42]

In another study, deep freezer storage (–80 °C) for two months also seemed to have a negative impact upon the antioxidant capacity and the oxidation status of stored EBM.[44] Again, the storage of EBM at – 80 C was not recommended by the authors as optimal conditions to store EBM for two months.

Grey literature

Relevant grey literature was investigated regarding the impact of common temperature ranges on EBM in terms of contamination and nutritional quality. Existing grey literature guidelines and recommendations from Australia,[12] the UK (based upon the NHS recommendations),[53,54] and the USA,[55] were located. While these grey literature sources do not explicitly address the impact of particular temperatures on contamination and nutritional quality, guidelines and recommendations are made for safe storage. The 2012 NHMRC infant feeding guidelines already cited in the report is the benchmark for Australian grey literature and makes the following recommendations (see table 3).[12]

Table 3. Grey literature recommendations for EBM storage

Room temperature (26 °C or lower)	Refrigerator (5 °C or lower)	Freezer compartment in refrigerator (−15 °C)	Freezer with separate door (−18 °C)	Deep freezer (−20 °C)
6-8 hours[α]	72 hours maximum[β]	2 weeks maximum	3 months maximum[Ω]	6-12 months maximum

[α] The Baby Centre (UK) recommends no longer that six hours at 25 °C or lower.[53] The ARHQ in the United States notes that 3-4 hours at between 16 and 29 °C is optimal with 6-8 hours only recommended for 'very clean' conditions.[55]

[β] The ARHQ suggests that 72 hours at 4 °C is 'optimum' while EBM can be safely stored in 'very clean' conditions for up to five to eight days.[55]

[Ω] The NHS in the UK and other UK grey literature sources advise EBM stored at –18 °C or –20 °C is safe for up to six months.[54]

The NHMRC guidelines also recommend transporting EBM in an insulated container with a freezer brick but do not stipulate a time or temperature. A number of grey literature sources from the United Kingdom provide further guidance around storage and transport of EBM noting that EBM can be safely transported in between –4 °C and 2 °C.[53] The Academy of Breastfeeding Medicine Protocol Committee's protocol (published source cited in the present report) notes that insulated cooler bag storage should be safe for up to 24 hours.[11] This protocol is the bench mark grey literature source in the United States and is cited by the Agency for Healthcare Research and Quality (AHRQ) in their guidelines.[55] Grey literature sources generally concur that EBM should be stored at the rear of refrigerator where temperature is coldest. At –4°C the AHRQ recommends optimal storage for up to six months and that 12 months is 'acceptable'.[55]

Potential limitations of included studies

Pooling EBM

Two studies pooled EBM collected from multiple mothers in order to conduct experiments on a number of identical and equally-sized samples and to overcome limitations imposed by undertaking a wide range of biochemical analyses.[48,50] This may be a potential limitation regarding the transferability of results to the impact of storage of EBM from individual mothers.

Deep freezing EBM

Three reported upon 'deep-freezing' EBM at extremely low temperatures (–80 °C).[42,44,46] Such storage practices are unlikely to be common in clinical or home storage contexts.

Gap analysis

Transport

No studies were located that dealt explicitly with the transport of EBM in terms of its impact upon contamination and or nutritional quality.

Diseases caused by contaminated EBM

No studies were located that reported upon diseases caused by contaminated EBM as a result of storage or transport.

Context

All included studies were conducted as bench research in laboratory contexts. No studies were located that reported upon home, hospital or community settings.

Review Question 3: What is the evidence about disinfection and cleaning procedures for handling EBM?

Six papers were identified that met the selection criteria for Question 3 (see table 4). All six papers reported upon evidence around disinfection and cleaning procedures for handling EBM. Results for one paper were also included for Question 1.[20]

Table 4. Foci of included studies in relation to Question 3

Study	EBM equipment disinfection/cleaning	EBM expression methods	Hand/breast disinfection/cleaning	Context
Becker et al. 2011.[56]		Hand expression vs pump	√	
Karimi et al. 2012.[57]	Educational intervention		√	
Rhodes 2011.[58]	√		√	
Scott et al. 2010.[59]	Hospital practice improvement			
Engur et al. 2014.[60]	Case study of hospital practices		√	
Serra et al. 2013.[20]				√

Disinfection and cleaning methods (equipment)

Four studies examined disinfection and cleaning methods for EBM expression equipment such as breast pumps and bottles.[57-60] Karimi and colleagues investigated the impact of a brief educational intervention on contamination of EBM.[57] While the study did not go into great detail regarding the intervention, it was mentioned that information about cleaning and disinfecting equipment (containers) was given to mothers. The study only measured results (bacterial colonies in EBM) the day after the intervention and did not conduct a follow-up. Results indicated a significant reduction in the number of bacterial colonies in EBM and traced the main source of contamination to EBM containers and pumps.

Rhodes conducted a literature review and provided very clear and detailed instructions and recommendations for the disinfection and cleaning of EBM equipment.[58] While many of the sources come from beyond the field of lactation and infant feeding studies and instead draw upon evidence for hand and medical equipment disinfection and cleaning practices, the recommendations uphold and support those detailed in the recent (2012) NHMRC Infant Feeding Guidelines.[12]

Scott and colleagues conducted a literature review and developed a 'best practice' protocol for implementation in hospital settings around the disinfection and cleaning of infant feeding equipment.[59] While the study did not discuss outcomes related to contamination (e.g. infection or contamination rates), it did report upon barriers to implementation of disinfection and cleaning procedures for equipment around costs and staff perceptions about the extent that disinfection was necessary. This may indicate that education is required to inform staff (and families) about the risks of contamination from improperly cleaned EBM equipment and how it should be cleaned. An epidemiological case study has also been conducted and demonstrated the importance of disinfection and cleaning of EBM equipment due to its potential as a source of infection outbreak in hospital contexts.[60] The location of the equipment in the

hospital (in the neonatal intensive care unit), and staff and mothers' knowledge and practice of cleaning procedures were also cited as possible contributing factors behind the outbreak described.[60]

Expression methods

One study examined the differences between hand and pump expression methods.[56] Becker and colleagues conducted a systematic review locating two studies that found no difference in contamination or adverse effects between hand and pump EBM.[56]

Disinfection and cleaning methods (hands and or breasts)

Two studies examined disinfection and cleaning methods for hands and/or breasts.[56,58] Becker and colleagues located one study that reported that one-time use of a breast cleansing process with antibacterial soap reduced bacterial counts in the milk sample.[56] However, as the authors point out, the feasibility of using soap and anti-bacterial agents on the breasts six to eight times a day raises concerns both for the mothers' skin and willingness to continue this procedure for a length of time; there may also be concerns over residues of the antibacterial agent in EBM which may be detrimental to the probiotic actions of human milk for infants.

Rhodes provides detailed guidance and recommendations regarding hand and breast cleaning and disinfection practices based upon guideline and recommended practice sources cited in a literature review.[58] While many of the sources come from beyond the field of lactation and infant feeding studies and instead draw upon evidence for hand and medical equipment disinfection and cleaning practices, the recommendations uphold and support those detailed in the recent (2012) NHMRC Infant Feeding Guidelines.[12]

Engur and colleagues' case study mentions the importance of teaching mothers appropriate hand hygiene practices for the collection of EBM in hospitals and supporting such practices.[60]

Contexts

Two studies reported upon the differences and/or impact of context on disinfection and cleaning practices for handling EBM.[20,58] One study cited in a literature review reported a difference in contamination levels between homes and hospitals and found that EBM expressed at home has higher contamination levels.[58] Serra and colleagues also upheld this finding using an exploratory cross-sectional experimental design.[20]

Grey literature

Relevant grey literature was investigated for evidence about disinfection and cleaning procedures for handling EBM. In Australia, the NHMRC infant feeding guidelines is the benchmark for Australian grey literature.[12] Likewise, in the United Kingdom, the NHS provides clear recommendations for cleaning infant feeding equipment that comes into contact with EBM.[61] In summary, there are several ways to sterilise feeding equipment, including using a cold water sterilising solution, steam sterilising, or boiling. Guidance for cleaning bottles is the same for EBM expression and storage equipment. In United States, the Academy of Breastfeeding Medicine Protocol Committee protocol, cited by the ARHQ, recommends use of containers and pumping equipment that have been washed in hot, soapy water and rinsed.[11] Cleaning in a dishwasher is acceptable and dishwashers that additionally heat the water may be better. Boiling containers after washing is also recommended which is particularly important where the water supply may not be clean. The US Food and Drug Administration concurs with this and also recommends following any guidance provided by the manufacturers of the specific equipment. Clear instructions for cleaning and disinfecting EBM equipment is referenced in the present report (see the literature review by Rhodes in Appendix 5) and is upheld by the current relevant grey literature.[58]

Gap analysis

Education interventions

Little evidence was located that reported upon education interventions with mothers and or health care workers regarding techniques to minimise contamination via EBM equipment and/or expression methods. Further research in this area would be valuable based upon the positive results reported in one study.[57] Scott and colleagues discussed a 'best practice' implementation project and noted that education may be necessary for staff around EBM equipment disinfection processes.[59]

Storage devices

No studies were located that discussed disinfection or cleaning of EBM storage devices such as refrigerators, freezers or cool boxes (Eskys).

Developing Countries

It was noted by the reviewers that a number of studies examined or described disinfection and or cleaning procedures for handling EBM in the context of low-resource developing countries. These papers were outside the scope of this review and were not selected for potential inclusion.

Review Question 4: What is the evidence about operational procedures that can minimise the risk of EBM misdelivery?

There was a lack of research published since 2008 specifically addressing operational procedures that can minimise the risk of EBM misdelivery. Following the search, five studies were included for Question 4 and are described in table 5. All of the studies found were either 'low' or 'very low' quality, and only one study followed the traditional reporting structure of providing an introduction, methods, results and discussion.[15] This made it difficult to classify the study design and assign a level and quality of evidence ranking. There were three studies identified which specifically addressed bar coding as an operational procedure to reduce the risk of EBM misdelivery; however, as this process is not feasible in the current NSW environment, the results of these studies are not discussed.[62-64]

Table 5: Studies included for Question 4

Paper	Study methods (design, sample size, setting)	Misdelivery prevention method	Relevant Study findings	NHMRC Level of Evidence	Quality of evidence
Barbas et al. 2013[65]	Survey study of 120 participants, Boston USA	Establishment of mother's milk technicians	Survey response: 100% of respondents agreed that labelling was clear and easy to understand, while 85.12% agreed that mother's milk technicians improve safety of breast milk administration	N/A	Very low
Gabrielski et al. 2011[13]	Descriptive, narrative article reporting on one hospital's experiences in Colorado, USA (participants=N/A)	Milk Lab Project and bar coding	The authors state that this project has resulted in less errors, and that only one error has occurred out of 219,544 samples since introducing bar coding	N/A	Very low

Zeilhofer et al.2009[15]	Before and after study, 15 bed NICU, Switzerland	Critical incident monitoring and implementation of strategies based on incidents (i.e. changing labelling)	Despite introducing changes to labelling, administration errors did not reduce significantly	III – 3	Low
Drenckpohl et al. 2007[66]	Descriptive, narrative article reporting on one hospital's experiences in a 35 bed NICU Illinois, USA	Six Sigma Quality Improvement methodology, development of a new policy and education for staff	The authors state that since the introduction of the new policy there had been no incidents of milk being delivered to the wrong infant	N/A	Very low
Zhang et al. 2014[67]	Health care failure mode and effect analysis, unclear number of participants, Minnesota USA	Potential risks and strategy identification	'(a) finding dedicated EBM preparation space, (b) developing a staffing model to support milk technicians, and (c) creating a process for the electronic medical record to track feedings at the children's hospital.' (page 35)	N/A	Very low

The following factors were identified that increased the risk of misdelivery:

- When mothers and babies are separated or are in separate patient care areas.[68]
- Busy nurses.[65]
- Interruptions.[15]
- Non attention/distraction.[15]
- Similar sounding names.[15,66]
- Night time.[15]

Risk analysis and quality improvement activities

A risk analysis using the healthcare failure mode and effect analysis approach was conducted to quantify risks related to the human milk feeding process.[67] This risk analysis identifies current ways the process could result in errors, what interventions could be used to prevent errors, and which of these interventions are viewed as priorities to implement. This was performed by an interdisciplinary team. Based on the analysis, the authors recommended the following: '(a) finding dedicated EBM preparation space, (b) developing a staffing model to support milk technicians, and (c) creating a process for the electronic medical record to track feedings at the children's hospital.' (page 35).[67 p.35]

In a NICU in Illinois, USA, an evaluation of misappropriation of expressed breast milk was conducted following the Six Sigma quality improvement methodology.[66] This process employs 'data to reveal defects in

procedures, to employ customer-driven measures to establish the target for ideal performance, and ultimately to operate within six standard deviations of average performance' (page 163). Specifically, the quality improvement team use the five step method of defining, measuring, analysing, improving and controlling (DMAIC) to reduce errors. In this case it was found that there were inconsistent practices regarding breast milk collection, storage, preparation and administration. No policy existed to guide staff for handling and distributing breast milk. There was also no guidelines or information for parents or family members. Based on the findings of a root cause analysis, changes were made including standardising intake, storage, preparation, distribution and administration of expressed breast milk. A policy was developed and staff received education regarding its use. The authors state that the new policy was accepted with little resistance, and that in the period following its implementation, there were no cases of breast milk misappropriation.[66] The authors conclude that the use of Six Sigma methodology and the development of 'an appropriate and effective breast milk administration policy can lead to reduced misappropriation…staff must be consistent in following the policy and also must be aware of the seriousness to the infant and families of the NICU'.[66 p.166]

Zeilhofer et al. evaluated critical incident monitoring, which is a 'voluntary, anonymous, non-punitive reporting system of harmful and potentially harmful events' including analysis of reports and changes based on this analysis.[15 p.1277] During the period of critical incident monitoring labels were changed on the bottle twice. The first change saw the introduction of the name, date of birth, patient's ID and case number as well as bottle content whereas previously only a number corresponding to the neonate was present. As this did not result in a drop in administration errors, the labels were once again changed, with the addition of a 'big black bar.' No statistically significant changes were seen in the number of breast milk administration errors, although it should be noted that these errors were rare (range from 1-6 per year). The authors conclude that critical incident monitoring enabled the authors to recognise the problem of breast milk administration errors in their NICU.[15]

Operational procedures/ interventions

Mother's milk technicians are specialist trained milk technicians who are dedicated to maintaining freezers, refrigerators, and ensuring proper labelling of milk. The authors conclude that the role of a mother's milk technician ensures optimal quality control. However, this was a very low level study that did not specifically report on errors in delivering expressed milk.[65]

The milk lab project was established to achieve two main goals, the first being to 'standardise the storage, preparation, and administration of human milk in order to improve the safety and quality'. The second goal was to decrease nurses' time away from the patient's bedside and to increase their time with direct care activities.'[65 p.226] The human milk lab project involves the establishment of a dedicated room and workspace adequate to the needs of storing milk in terms of security, cooling, and ease of picking up and dropping off milk. The milk lab also includes bar coding and verification processes and is staffed by dedicated technicians. There are multiple scans of the bar code to ensure that the right patient receives the right milk throughout the storage, preparation and administration process. The authors report that the milk lab and bar coding reduce errors in milk administration.[65]

Recommendations from literature reviews

Due to the poor quality of the included articles, literature reviews were also evaluated to determine whether any guidance was provided in these documents to reduce the risk of EBM misdelivery. Two literature reviews were included. There was no evidence that these reviews were conducted using a rigorous or systematic development strategy, and they appeared to be driven largely by the author's own expert opinion.

The O'Malley literature review recommended that milk storage/collection containers must be labelled adequately, including the patient name and date of expression.[10] Containers should be stored in a 'per patient section of either the refrigerator or freezer'.[10 p.21]

A literature review suggested the following strategies to ensure that the right expressed breast milk was delivered to the right baby, some of which were informed by the policy from NSW Health:[68,69]

- Only separate mothers and babies when clinically indicated
- When separated, ensure there is a 'highly reliable, organization-specific identification process.'[68p.55]
- Ensuring identification bands for both the infant and mother are complete with all details and legible.
- Breast milk containers should be labelled 'consistently, correctly, and clearly, using moisture-resistant ink.'[68 p.55]
- Both the infant and their mother's name should be included on the label, in addition to the infant's 'medical record number, the date and time the milk was expressed, and the date and time the milk was thawed.'[68 p.55]
- When the mother is expressing, provide and empty breast milk container and completed label and explain how to document the date and time the milk was expressed on the label. Display how to apply the label onto the container to ensure the information is visible when opening the container.
- Prior to storage the label should be checked for 'accuracy, legibility, and completeness of information.'[68 p.55]
- In terms of storage and management, a separate designated area should be established for refrigerators and freezers storing breast milk. Within each refrigerator or freezer, an allocated separate area should exist for each infant's labelled container.
- A current log should be kept with the mother's name, the date and time breast milk was expressed and when milk was dispensed for the infant.
- The mother should verify the breast milk container label 'matches the mother's and baby's identification bands.'[68 p.55]
- Educational opportunities should be provided to staff on safe and correct handling of breast milk, patient should be given written and verbal information, a process to ensure competency for agency/floating/temporary staff should be in place.

Gap analysis

There is a significant lack of high quality research that establishes a causal link between an intervention or process and the rate of EBM misdelivery. The overall quality of the literature on the topic is very low, with the majority of articles consisting of descriptive reports providing an overview of current practice in one setting. To truly determine the effectiveness of strategies to reduce errors of breast milk delivery, randomised controlled trials or other experimental studies are required. No evidence was found regarding the effectiveness of individual patient (baby) bedside refrigerators for reducing the risk of EBM misdelivery. The majority of the literature focused on providing a dedicated individual space per patient in a shared refrigerator. However, it stands to reason that individual patient bedside refrigerators could be an appropriate mechanism to reduce the risk of EBM misdelivery if this approach is deemed feasible.

5 Discussion

Implications for policy

The current policy developed by NSW Health (Maternity – Breast Milk: Safe Management) is a comprehensive document that currently reflects many of the findings and recommendations from the literature.[69]

Regarding the storage and transport of EBM as well as the handling, cleaning and disinfection practices surrounding its collection and storage, the latest NHMRC infant feeding guidelines are accurate and in concurrence with the evidence located in this review.[12]

In individual units where misdelivery of EBM is occurring, healthcare professionals may like to consider implementing quality improvement initiatives and risk analysis to implement strategies (potentially in line with the NSW Health policy) to reduce this risk.

Applicability of findings to NSW context

Much of the included research literature addressed issues of relevance to breastfeeding over a period of time, and did not report on a single exposure through misdelivery of EBM to the wrong baby. Within the HIV research literature, techniques such as heat treatment of EBM has been reported to be a simple and cost-effective approach to decrease vertical HIV transmission in less resourced contexts, but would not be advocated within the NSW context, where HIV positive women are advised not to give breast milk to their baby.

The majority of findings around the storage of EBM, disinfection of equipment and cleaning practices around expression and storage practices are applicable in the NSW context. While deep freezing at –80 °C is likely beyond the capacity of most clinical, community and private contexts, existing recommendations for refrigeration and freezing stored EBM remain safe and valid.

Although studies were found relating to bar coding, this approach is currently not feasible in the NSW context.

There were some low level findings relating to the effectiveness of dedicated milk technicians and milk labs to improve quality control of milk delivery. The introduction of milk technicians was reported to result in less errors and relieve the burden of these tasks from nurses. It is not currently clear whether the establishment of this position is feasible within the NSW context.

Recommendations

Risks of pathogen transmission from mother to child

The current NHMRC infant feeding guidelines, and the NSW Health policy (Maternity – Breast Milk: Safe Management) adequately reflects the recommendations within the included literature regarding risks of pathogen transmission from mother to child.[12] Benefits for the infant to continue receiving EBM must be considered against the possible severity of the disease. There are few pathogens with clear evidence of transmission through breast milk that cause significant morbidity and mortality, but in these cases the administration of breast milk should be avoided (such as with HIV).

The potential risk for transmission of pathogens through EBM misdelivery, although extremely low, is considered a hospital error and poses a risk to patient safety.

Impact of storage and transport of EBM

The current NHMRC infant feeding guidelines and other grey literature sources adequately reflect the recommendations within the included literature around the impact of storage on EBM.[12] In summary, EBM can be safely stored at –20 °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 °C may an option for moderate term (up to two months) storage of EBM (as opposed to long term storage e.g. over 2 months).

Healthworkers and families should be aware that the thermostat setting on commercial refrigeration devices may not accurately represent the actual internal temperature of the unit over time.

The type of container that EBM is stored in seems to have a very slight impact upon bactericidal activity and nutrition of EBM. It may be advisable to choose Pyrex or hard polypropylene containers over soft plastic (polyethylene) bags due to the reduced likelihood of puncture and spillage.

Disinfection and cleaning procedures

As the bacteriological profile of EBM at the time of expression seems to directly influence the composition of EBM tested after storage, adequate cleaning and disinfection of EBM expression and storage equipment should be performed in all contexts. This is especially necessary for shared equipment. Again, the NHMRC infant feeding guidelines and other grey literature sources adequately reflects the recommendations within the included literature around the disinfection and cleaning of EBM collection equipment and hygienic expression practices.[12]

EBM misdelivery

The current NSW Health policy (Maternity – Breast Milk: Safe Management) adequately reflects the recommendations within the literature to reduce the risk of misdelivery of EBM.[69] Continuous education, training and monitoring of the policy is recommended.

Although it may not be feasible at this point in time, bar coding processes and the establishment of dedicated milk technicians may be considered and discussed in the future.

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8 Appendices

Appendix 1. Search strategy and results of the search

Search Strategies for databases

MEDLINE (PubMed Platform)

Question 1: What are the risks of pathogen transmission from mother to child?

Search	Query
#1	breast milk [tw] OR human milk [tw]
#2	express* [tw]
#3	breast milk expression [mh]
#4	bacteria [tw] OR human immunodeficiency virus [tw] OR HIV [tw] OR cytomegalovirus [tw] OR hepatitis B [tw] OR HBV [tw] OR hepatitis C [tw] OR HCV [tw] OR Human T cell leukaemia virus type 1 [tw] OR Human T cell leukemia virus type 1 [tw] OR HTLV1 [tw] OR Human T cell leukaemia type 2 [tw] OR Human T cell leukemia type 2 [tw] OR HTLV2 [tw] OR herpes simplex virus type 1 [tw] OR HSV1 [tw] OR herpes simplex virus type 2 [tw] OR HSV2 [tw] OR rubella [tw] OR syphilis [tw] OR varicella zoster virus [tw] OR VZV [tw]
	[[#1 AND #2] OR #3] AND #4
Limits	Publication date from 2008/01/01 to current; English language.

Question 2: What is the impact of storage and transport of EBM on contamination and nutritional quality of milk?

Search	Query
#1	breast milk [tw] OR human milk [tw]
#2	express* [tw]
#3	breast milk expression [mh]

#4	storage [tw] OR refrigerat* [tw] OR freez* [tw] OR container [tw] OR bag [tw] OR temperature [tw] OR fridge [tw] OR cooler [tw]
#5	transport* [tw]
#6	contaminat* [tw] OR postnatal infection [tw] OR postnatal transmission [tw]
#7	nutrient* [tw] OR nutrition* [tw]
	[[#1 AND #2] OR #3] AND [#4 OR #5] AND [#6 OR #7]
Limits	Publication date from 2008/01/01 to current; English language.

Question 3: What is the evidence about disinfection and cleaning procedures for handling EBM?

Search	Query
#1	breast milk [tw] OR human milk [tw] OR breast milk expression [mh]
#2	express* [tw]
#3	breast milk expression [mh]
#4	container [tw] OR bag [tw] OR bottle [tw] OR teat [tw] OR syringe [tw] OR refrigerat* [tw] OR freez* [tw] OR fridge [tw] OR cooler [tw] OR cap [tw] OR breast pump* [tw] OR hand expression [tw]
#5	clean* [tw] OR wash* [tw] OR disinfect* [tw] OR steril* [tw] OR pasteuri* [tw] OR cleans* [tw] OR hygien* [tw] OR handling [tw] OR treatment* [tw] OR process* [tw] OR procedure* [tw] OR method* [tw]
	[[#1 AND #2] OR #3] AND [#4 OR #5]
Limits	Publication date from 2008/01/01 to current; English language.

Question 4: What is the evidence about operational procedures that can minimise the risk of EBM misdelivery?

Search	Query
#1	breast milk [tw] OR human milk [tw]
#2	express* [tw]
#3	breast milk expression [mh]

#4	misdeliver* [tw] OR label* [tw] OR dispens* [tw] OR identif* [tw] OR registrat* [tw] OR band [tw] OR tag [tw] OR medical record number [tw] OR collect* [tw] OR check* [tw] OR manag* [tw] OR screen* [tw] OR system [tw] OR record* [tw] OR education* [tw]
	[[#1 AND #2] OR #3] AND #4
Limits	Publication date from 2008/01/01 to current; English language.

CINAHL

Question 1: What are the risks of pathogen transmission from mother to child?

Search	Query
#1	TX "breast milk" OR TX "human milk"
#2	TX "express" OR TX "expressing" OR TX "expressed" OR TX "expression"
#3	MH milk expression OR MH milk, human
#4	TX "bacteria" OR TX "human immunodeficiency virus" OR TX "HIV" OR TX "cytomegalovirus" OR TX "hepatitis B" OR TX "HBV" OR TX "hepatitis C" OR TX "HCV" OR TX "Human T cell leuk#emia virus type 1" OR TX "HTLV1" OR TX "Human T cell leuk#emia type 2" OR TX "HTLV2" OR TX "herpes simplex virus type 1" OR TX "HSV1" OR TX "herpes simplex virus type 2" OR TX "HSV2" OR TX "rubella" OR TX "syphilis" OR TX "varicella zoster virus" OR TX "VZV"
	[[#1 AND #2] OR #3] AND #4
Limits	Publication date from 2008/01/01 to current; English language.

Question 2: What is the impact of storage and transport of EBM on contamination and nutritional quality of milk?

Search	Query
#1	TX "breast milk" OR TX "human milk"
#2	TX "express" OR TX "expressing" OR TX "expressed" OR TX "expression"
#3	MH milk expression OR MH milk, human
#4	TX "storage" OR TX "refrigerat*" OR TX "freez*" OR TX "container#" OR TX "bag#" OR TX "temperature#" OR TX "fridge#" OR TX "cooler#" OR TX "transport*"
#5	TX "contaminat*" OR TX "postnatal infection" OR TX "postnatal transmission" OR TX "nutrient*" OR TX "nutrition*"

	[[#1 AND #2] OR #3] AND [#4 OR #5]
Limits	Publication date from 2008/01/01 to current; English language.

Question 3: What is the evidence about disinfection and cleaning procedures for handling EBM?

Search	Query
#1	TX "breast milk" OR TX "human milk"
#2	TX "express" OR TX "expressing" OR TX "expressed" OR TX "expression"
#3	MH milk expression OR MH milk, human
#4	TX "container" OR TX "bag" OR TX "bottle" OR TX "teat" OR TX "syringe" OR TX "refrigerat*" OR TX "freez*" OR TX "fridge" OR TX "cooler" OR TX "cap" OR TX "breast pump*" OR TX "hand expression"
#5	TX "clean*" OR TX "wash*" OR TX "disinfect*" OR TX "steril*" OR TX "pasteuri*" OR TX "cleans*" OR TX "hygien*" OR TX "handling" OR TX "treatment*" OR TX "process*" OR TX "procedure*" OR TX "method*"
	[[#1 AND #2] OR #3] AND [#4 OR #5]
Limits	Publication date from 2008/01/01 to current; English language.

Question 4: What is the evidence about operational procedures that can minimise the risk of EBM misdelivery?

Search	Query
#1	TX "breast milk" OR TX "human milk"
#2	TX "express" OR TX "expressing" OR TX "expressed" OR TX "expression"
#3	MH milk expression OR MH milk, human
#4	TX "misdeliver*" OR TX "label*" OR TX "dispens*" OR TX "identif*" OR TX "registrat*" OR TX "band" OR TX "tag" OR TX "medical record number" OR TX "collect*" OR TX "check*" OR TX "manag*" OR TX "screen*" OR TX "system" OR TX "record" OR TX "education"
	[[#1 AND #2] OR #3] AND #4
Limits	Publication date from 2008/01/01 to current; English language.

EMBASE

Question 1: What are the risks of pathogen transmission from mother to child?

Search	Query
#1	"breast milk"/de OR "breast milk":ti,ab OR "breast milk"/syn
#2	"express"/de OR "expressing"/de OR "expressed"/de OR "expression"/de
#3	"breast milk expression"/de OR "breast milk expression":ti,ab OR "breast milk expression"/syn
#4	"bacteria"/de OR "human immunodeficiency virus"/de OR "HIV"/de OR "cytomegalovirus"/de OR "hepatitis B"/de OR "HBV"/de OR "hepatitis C"/de OR "HCV"/de OR "Human T cell leuk#emia virus type 1"/de OR "HTLV1"/de OR "Human T cell leuk#emia type 2"/de OR "HTLV2"/de OR "herpes simplex virus type 1"/de OR "HSV1"/de OR "herpes simplex virus type 2"/de OR "HSV2"/de OR "rubella"/de OR "syphilis"/de OR "varicella zoster virus"/de OR "VZV"/de
	[[#1 AND #2] OR #3] AND #4
Limits	Publication date from 2008/01/01 to current; English language.

Question 2: What is the impact of storage and transport of EBM on contamination and nutritional quality of milk?

Search	Query
#1	"breast milk"/de OR "breast milk":ti,ab OR "breast milk"/syn
#2	"express"/de OR "expressing"/de OR "expressed"/de OR "expression"/de
#3	"breast milk expression"/de OR "breast milk expression":ti,ab OR "breast milk expression"/syn
#4	"storage"/de OR "refrigeration"/de OR "refrigerate" OR "freezer" OR "freezing"/de OR "container"/de OR "bag"/de OR "temperature"/de OR TX "fridge"/de OR "cooler"/de OR "transport"/de
#5	"contamination"/de OR "postnatal infection"/de OR "postnatal transmission"/de OR "nutrient"/de OR "nutrition"/de
	[[#1 AND #2] OR #3] AND [#4 OR #5]
Limits	Publication date from 2008/01/01 to current; English language.

Question 3: What is the evidence about disinfection and cleaning procedures for handling EBM?

Search	Query
#1	"breast milk"/de OR "breast milk":ti,ab OR "breast milk"/syn
#2	"express"/de OR "expressing"/de OR "expressed"/de OR "expression"/de
#3	"breast milk expression"/de OR "breast milk expression":ti,ab OR "breast milk expression"/syn
#4	"container"/de OR "bag"/de OR "bottle"/de OR TX "teat"/de OR "syringe"/de OR "refrigerator"/de OR "refrigerating"/de OR "freezer"/de OR "freezing"/de OR "fridge"/de OR "cooler"/de OR "cap"/de OR "breast pump"/de OR "hand expression"/de
#5	"clean"/de OR "cleaning"/de OR "wash"/de OR "washing"/de OR "disinfect"/de OR "disinfecting"/de OR "disinfection"/de OR "sterilise"/de OR "sterilise"/de OR "sterilising"/de OR "sterilizing"/de OR "sterilisation"/de OR "sterilisation"/de OR "pasteurise"/de OR "pasteurize"/de OR "pasteurising"/de OR "pasteurizing"/de OR "pasteurisation"/de OR "pasteurization"/de OR "cleanse"/de OR "hygiene"/de OR "hygienic"/de OR "handling"/de OR "treatment"/de OR "process"/de OR "procedure"/de OR "method"/de
	[[#1 AND #2] OR #3] AND [#4 OR #5]
Limits	Publication date from 2008/01/01 to current; English language.

Question 4: What is the evidence about operational procedures that can minimise the risk of EBM misdelivery?

Search	Query
#1	"breast milk"/de OR "breast milk": ti,ab OR "breast milk"/syn
#2	"express"/de OR "expressing"/de OR "expressed"/de OR "expression"/de
#3	"breast milk expression"/de OR "breast milk expression":ti,ab OR "breast milk expression"/syn
#4	"misdelivery"/de OR "misdelivering"/de OR "label"/de OR "labeling"/de OR "labelling"/de OR "dispense"/de OR "dispensing"/de OR "dispensary"/de OR "identification"/de OR "identifying"/de OR "identify"/de OR "registrate"/de OR "registrating"/de OR "registration"/de OR "band"/de OR "tag"/de OR "tagging"/de OR "medical record number"/de OR "collect"/de OR "collecting"/de OR "collection"/de OR "check"/de OR "checking"/de OR "manage"/de OR "managing"/de OR "management"/de OR "screen"/de OR "screening"/de OR "system"/de OR "record"/de OR "education"/de
	[[#1 AND #2] OR #3] AND #4
Limits	Publication date from 2008/01/01 to current; English language.

JBI Database of Systematic Reviews and Implementation Reports

Question 1: What are the risks of pathogen transmission from mother to child?

Search	Query
#1	breast milk OR human milk
#2	express OR expressing OR expressed OR expression
#3	bacteria OR human immunodeficiency virus OR HIV OR cytomegalovirus OR hepatitis B OR HBV or hepatitis C OR HCV OR Human T cell leukemia virus type 1 OR HTLV1 OR Human T cell leukemia type 2 OR HTLV2 OR herpes simplex virus type 1 OR HSV1 OR herpes simplex virus type 2 OR HSV2 OR rubella OR syphilis OR varicella zoster virus OR VZV
	#1 AND #2 AND #3
Limits	Publication date from 2008/01/01 to current; English language.

Question 2: What is the impact of storage and transport of EBM on contamination and nutritional quality of milk?

Search	Query
#1	breast milk OR human milk
#2	express OR expressing OR expressed OR expression
#3	storage OR refrigeration OR freezing OR temperature OR transport
#4	contamination OR bacteria OR virus OR postnatal infection OR postnatal transmission OR nutrient OR nutrition
	#1 AND #2 AND [#3 OR #4]
Limits	Publication date from 2008/01/01 to current; English language.

Question 3: What is the evidence about disinfection and cleaning procedures for handling EBM?

Search	Query
#1	breast milk OR human milk
#2	express OR expressing OR expressed OR expression

#3	container OR bag OR bottle OR teat syringe OR refrigerator OR freezer OR fridge OR cooler OR cap OR breast pump OR hand expression
#4	clean OR wash OR disinfect OR sterilise OR pasteurize OR cleanse OR hygiene OR handling OR treatment OR process OR procedure OR method
	#1 AND #2 AND [#3 OR #4]
Limits	Publication date from 2008/01/01 to current; English language.

Question 4: What is the evidence about operational procedures that can minimise the risk of EBM misdelivery?

Search	Query
#1	breast milk OR human milk
#2	express OR expressing OR expressed OR expression
#3	misdelivery OR misdelivering OR label OR labeling OR labelling OR dispense OR dispensing OR dispensary OR identification OR identifying OR identify OR registrate OR registrating OR registration OR band OR tag OR tagging OR medical record number OR collect OR collecting OR collection OR check OR checking OR manage OR managing OR management OR screen OR screening OR system OR record OR education
	#1 AND #2 AND #3
Limits	Publication date from 2008/01/01 to current; English language.

Search Results

Question 1

The following search terms were added to the primary search terms: bacteria, human immunodeficiency virus, cytomegalovirus, hepatitis B, hepatitis C, human T cell leukaemia virus type 1 and 2, herpes simplex virus type 1 and 2, rubella, syphilis, and varicella zoster virus.

Using the formulated search strategy for research Question 1, the following numbers of articles were retrieved from the included databases:

- Medline (Pubmed): 108 articles
- CINAHL: 573 articles
- EMBASE: 61 articles
- Joanna Briggs Institute Library: 0 articles

In total, 742 articles were found across the four databases. Of these, 15 were duplicates; 727 articles were retrieved for title and abstract examination and imported into Endnote.

Question 2

The following search terms were added to the primary search terms: storage, refrigeration, freezer, container, bag, temperature, fridge, cooler, and transport, contamination, postnatal infection, postnatal transmission, nutrient and nutrition.

The search strategy for Question 2 located the following articles:

- Medline (Pubmed): 27 articles
- CINAHL: 1515 articles
- EMBASE: 159
- Joanna Briggs Institute Library: 2 articles

In total of 1703 articles were found across the four databases, with 18 duplicates. The title and abstracts of 1685 articles were imported into Endnote for further examination.

Question 3

The following search terms were added to the primary search terms: container, bag, bottle, teat, syringe, refrigeration, freezer, fridge, cooler, cap, breast pump, hand expression, clean, wash, disinfect, sterilisation, pasteurisation, cleanse, hygiene, handling, treatment, process, procedure, and method.

The search strategy for Question 3 located the following articles:

- Medline (Pubmed): 416 articles
- CINAHL: 1595 articles
- Embase: 331 articles
- Joanna Briggs Institute Library: 0 articles

In total, 2342 articles were identified with 187 duplicates; 2155 titles and abstracts were retrieved for inspection in Endnote.

Question 4

The following search terms were added to the primary search terms: misdelivery, labelling, dispensing, identification, registration, band, tag, medical record number, collect, check, management, screening, system, recording, and education.

The search strategy for Question 4 located the following articles:

- Medline (Pubmed): 352 articles

- CINAHL: 1513
- EMBASE: 428 articles
- Joanna Briggs Institute Library: 13 articles

A total of 2306 articles were located for Question 4. Among these, 203 articles were duplicates. The title and abstract of 2103 articles were imported into Endnote for further examination.

Across all searched databases and questions, after duplicates had been removed, 2830 potentially relevant articles were retrieved for title and abstract examination.

Screening articles

The titles and abstracts of retrieved articles were scanned by four reviewers to determine eligibility for full-text examination. Titles and abstracts were screened for the presence of key words in relation to the four focus questions of this review. Overall, 109 articles were selected for full-text examination.

- Question 1: 51 articles selected (bacteria: 51, HIV: 17: CMV: 10, HBV: 1, HCV: 1, HTLV I & II: 3, HSV 1 & 2: 2, varicella: 1, syphilis: 1.)
- Question 2: 31 articles selected. Three additional articles selected in Question 1 library.[51,52,70]
- Question 3: 19 articles selected. One additional article was selected in Question 1 library and was also included in the results for that question.[20]
- Question 4: Eight articles selected.

Selecting articles

The full texts of selected articles were scanned by four reviewers to determine eligibility for inclusion the review in terms of their adherence to the review's selection criteria (see Appendix 2. Full text articles were screened in relation to the four focus questions of this review. Overall, 31 articles were located by the searches and selected for inclusion.

- Question 1: 20 full text articles located and included: (bacteria,[2,18-20] HIV, [21,23,24] CMV, [25-28] HBV,[29] HCV,[30] HTLV I & II = 0, HSV 1 & 2 = 0, rubella = 0, varicella zoster virus = 0, syphilis = 0,). Eight full text studies could not be located: (Bacteria,[71-73] HIV,[74,75] CMV,[76,77] HCV (abstract),[38]).
- Question 2: Nine full text articles located and included.[41-47,51,52] One full text article from the Question 1 search was unable to be located.[70] One study was not conducted in an OECD country, however due to the similarity of the study to the other included studies, a decision was made to retain the study for inclusion.[43]
- Question 3: Five full text articles located and included.[20,56,57,59,78] One further study could not be located in full text.[79]
- Question 4: Three full text articles located and included.[15,65,67]

Reference snowballing

The reference lists of articles selected at full text in Phase 3 were inspected for additional potentially relevant studies in relation to the four focus questions of this review. Overall, 12 articles were selected for inclusion from reference snowballing.

- Question 1: Six additional articles were included from the reference lists of included articles: (bacteria,[16,17] HIV,[22] HCV,[31] HTLV I &I I,[33] rubella,[34]).
- Question 2: Three additional articles were included from the reference lists of included articles.[48-50]
- Question 3: One articles was included from the reference lists of included articles.[60]
- Question 4: Two additional articles were identified from the reference lists of included articles.[13,66]

Appendix 2. Scope of the review and selection criteria

Selection criteria for the review

Selection criteria for this review were developed according to each review question and are reported below. Overall selection criteria were:

- Context: Included articles must contain evidence pertaining to at least one identified context including: homes, hospitals and the community. Community was broadly defined to include places other than home where the mother may spend time during the day, such as the workplace or support organisation for new mothers.
- Feeding mode: articles must contain evidence relevant to EBM; evidence related to breastfeeding was excluded.
- Literature on milk banking is excluded from the review.
- Year: Papers published in or after 2008 were eligible for inclusion in this review. Important papers published prior to this date were able to be included if deemed by the review team to be essential. One paper published prior to 2008 was included for Question 4.[66]

Scope for Question 1

This question focuses upon risks around pathogen contamination of EBM and misdelivery to the wrong baby. The sources of contamination of EBM that are of interest are viruses and bacteria, some of which may enter the EBM via maternal transmission. Pathogens of interest in this review are those identified in NSW Health's policy directive "Maternity – Breast Milk: Safe Management". [69] They are:

- Bacteria, particularly normal skin flora
- Human Immunodeficiency Virus (HIV)
- Cytomegalovirus (CMV)
- Hepatitis B (HBV)
- Hepatitis C (HCV)
- Human T cell leukaemia virus type I (HTLV I)
- Human T cell leukaemia virus type II (HTLV II)
- Herpes simplex virus types I & 2 (HSV I & II)
- Rubella
- Syphilis
- Varicella Zoster Virus (VZV)

Once these pathogens have entered EBM they may be delivered either to the right or the wrong baby, leading to different outcomes.

The risk of transmission depends on whether or not the milk comes from the baby's own mother, and this distinction is crucial to determine the risks associated with misdelivery. This review specifies whether the results apply to EBM delivered to the right or wrong baby, or if the difference in risk between the two cases is not addressed in the literature.

This question focuses solely on the transmission of the above pathogens. The transmission of medicinal or recreational drugs from mother to babies is beyond the scope of this review.

Selection criteria for Question 1 were:

Pathogens of interest: Bacteria, particularly normal skin flora, Human Immunodeficiency Virus (HIV), Cytomegalovirus (CMV), Hepatitis B (HBV), Hepatitis C, (HCV), Human T cell leukaemia virus type I (HTLVI), Human T cell leukaemia virus type II (HTLVII), Herpes simplex virus types I & 2 (HSV I&II), Rubella, Syphilis, Varicella Zoster Virus (VZV). Other pathogens were excluded.

Scope for Question 2

This review question examines the impact of storage and transport on contamination and the nutritional quality of EBM.

Temperature storage patterns are defined as "length of time at a nominated temperature" and can be influenced by things such as; relocation or transport of EBM, location of EBM inside a cooling device such as a fridge or cooler, opening the cooling device or its contents. Each of these things may cause temperature variation in stored or transported EBM.

Storage methods for EBM that will be examined are fridge and cooler storage. Other commonly used storage methods will be reported upon if evidence is located.

Outcomes from this question are disease caused by contaminated EBM and the nutritional value of EBM as affected by storage and transport.

This question will examine storage and transport across three settings; homes, hospitals and community settings. Community settings are broadly defined and include places other than home where the mother may spend time during the day, such as the workplace or support organisation for new mothers.

Selection criteria for Question 2 were:

Included papers had to report upon transport or storage of EBM.

Transport: Included papers had to report upon activities resulting in relocation of EBM.

Storage: Included papers had to report upon common methods of EBM storage e.g. refrigerators, freezers, and coolers. Papers that reported upon the type of container used to store EBM were also retrieved following discussion with the funding organisation.

Temperature exposure: Included papers had to report upon the temperature of EBM transport or storage.

Outcomes: Included papers had to report upon bacterial or viral contamination, and/or nutritional quality of EBM in relation to its transport or storage.

Scope for Question 3

This review question examines procedures that can be set in place to prevent EBM being contaminated via improper handling. This question focuses upon differences in equipment, defined as bottles, teats and pumps, and upon differences in milk expression procedures, for example pump or hand expression.

Procedures that can be implemented to prevent EBM contamination via improper handling via equipment or procedures will be sought and examined.

This question will examine equipment and procedures across three settings; homes, hospitals and community settings. Community settings are broadly defined as in Question 2 and include places other than home where the mother may spend time during the day, such as the workplace or support organisation for new mothers.

Evidence from existing guidelines will be drawn upon if no other evidence exists.

Selection criteria for Question 3 were:

Disinfection/cleaning procedures: Included papers had to report upon disinfection and cleaning procedures for expressing, delivering and/or storing EBM equipment. The methods for disinfection/cleaning were not restricted.

Equipment: Included papers had to report upon equipment used with EBM e.g. bottles, teats, breast pumps, refrigerators.

Procedures: Included papers had to report upon EBM expression procedures e.g. breast pumps or hand expression.

Scope for Question 4

This review question examines the evidence about operational procedures around the risks of EBM misdelivery. This question will identify evidence on the risks of misdelivery as well as the procedures that minimise this risk.

Only evidence on procedures that can reasonably be implemented in the current NSW environment will be sought. New technologies such as bar coding, new software or electronic health record systems are beyond the scope of this question.

Selection criteria for Question 4 were:

Risks: Included papers had to report upon evidence around the risk of delivery of EBM to the wrong baby.

Procedures: Included papers had to report upon procedures that can minimise the risk of delivery of EBM to the wrong baby that can reasonably be implemented in the current NSW environment. Evidence that discussed new technologies such as bar coding, new software systems and/or electronic health record systems were excluded.

Appendix 3. Quality of evidence

A measure of the quality of evidence has been assigned to each included paper to reflect how well the studies were conducted in order to eliminate bias. This includes how study participants were selected, allocated to groups, treated, and how study outcomes were measured. Each included paper has been assessed in terms of its quality using the following critical appraisal criteria adapted from the Joanna Briggs Institute's approach to critical appraisal.

Systematic Reviews

1. Is the review question clearly and explicitly stated?

   Yes/No/Unclear/Not Applicable

2. Was the search strategy appropriate?

   Yes/No/Unclear/Not Applicable

3. Were the inclusion criteria appropriate for the review question?

   Yes/No/Unclear/Not Applicable

4. Were there methods used to minimise error in data extraction?

   Yes/No/Unclear/Not Applicable

5. Were the methods used to combine studies appropriate?

   Yes/No/Unclear/Not Applicable

Experimental Studies

1. Was there appropriate randomisation?

   Yes/No/Unclear/Not Applicable

2. Was allocation concealed?

   Yes/No/Unclear/Not Applicable

3. Were participants blinded to group allocation?

   Yes/No/Unclear/Not Applicable

4. Were the control and treatment groups comparable at entry?

Yes/No/Unclear/Not Applicable

5. Are confounding factors identified and strategies to deal with them stated?

Yes/No/Unclear/Not Applicable

Cohort and Case Control Studies

1. Has bias been minimised in relation to selection of cases and controls?

Yes/No/Unclear/Not Applicable

2. Have groups been treated identically other than the named intervention or exposure?

Yes/No/Unclear/Not Applicable

3. Are outcomes assessed using objective criteria?

Yes/No/Unclear/Not Applicable

4. Are outcomes measured in a reliable way?

Yes/No/Unclear/Not Applicable

5. Are confounding factors identified and strategies to deal with them stated?

Yes/No/Unclear/Not Applicable

Studies that are assigned a 'yes' for all five items will receive an evidence quality rating of 'very high'.

Studies assigned four 'yes' answers will be rated as 'high' quality.

Studies assigned three 'yes' answers will be rated as 'moderate' quality.

Studies assigned two 'yes' answers will be rated as 'low' quality.

Studies assigned one or no 'yes' answers will be rated as 'very low' quality.

Appendix 4. NHMRC levels of evidence hierarchy

Level	Intervention
I	A systematic review of level II studies
II	A randomised controlled trial
III-1	A pseudo-randomised controlled trial (i.e. alternate allocation or some other method)
III-2	A comparative study with concurrent controls: • Non-randomised, experimental trial • Cohort study • Case-control study • Interrupted time series with a control group
III-3	A comparative study without concurrent controls: • Historical control study • Two or more single arm study • Interrupted time series without a parallel control group
IV	Case series with either post-test or pre-test/post-test outcomes

Table adapted from NHMRC additional levels of evidence and grades for recommendations for developers of guidelines (https://www.nhmrc.gov.au/_files_nhmrc/file/guidelines/developers/nhmrc_levels_grades_evidence_120423.pdf)

Appendix 5. Characteristics and findings from included studies

Review Question 1: *What are the risks of pathogen transmission from mother to child?*

Bacteria

Study 1.	
Study	Civardi E, Garofoli C, Tzialla P, et al. 2013. Micro-organisms in human milk: lights and shadows.[2]
NHMRC Level of Evidence	IV Literature review
Objectives	To determine whether the non-sterility of breast milk is a protective factor, or rarely seen as a risk factor for the newborn.
Population	-
Methods	Literature review
Main results relevant to question	Human milk is a physiological source of bacteria to the infant gut, where it may play a variety of anti-infectious, anti-inflammatory, immunomodulatory and metabolic roles. Mastitis is a common disease (prevalence of 2–33%) of lactating women. In infective mastitis, *S. aureus* and *S. albus* are most common organisms; *E. coli* and *streptococci* are less frequent. Treatment with antibiotic therapy and breastfeeding or expression of breast milk should be continued. Contamination of expressed breast milk: Especially in neonatal intensive care units, contamination of EBM is an important issue. Reports suggest that EBM is not sterile; 800 analysed samples reported that only 22% showed no bacterial growth, with 87% being colonized with staphylococci. Following Holder pasteurisation, 93% were free of bacteria.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to question	In most cases the potential benefits of breast milk outweigh the possible negative effects and breastfeeding is not contraindicated.

Summary of limitations	-
Reviewer summary of evidence	Human milk may be a source of pathogenic micro-organism during maternal infection, or if contaminated during expression or in case of maternal vaccination.
Quality of evidence	Very low
Study 2.	
Study	Widger J, O'Connell N, Stack T. 2010. Breast milk causing neonatal sepsis and death.[16]
NHMRC Level of Evidence	IV Case study
Objectives	Three cases of late-onset neonatal sepsis, including one that resulted in death, occurring in preterm infants.
Population	Preterm infants: twins at 32 weeks gestation; singleton at 25 weeks gestation.
Methods	Three case studies in hospital
Main results relevant to question	This case study reports on three cases of late-onset neonatal septicaemia, including one which resulted in death, which were likely to have been caused by contaminated expressed breast milk.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to question	Breast milk can occasionally transmit serious viral and bacterial diseases to preterm infants. Routine screening and pasteurisation of breast milk administered to preterm infants should be considered. Late-onset neonatal sepsis is classically acquired from the care-giving environment, but may also be from contaminated maternal breast milk.
Summary of	-

limitations	
Reviewer summary of evidence	Uncertain/not known if results apply to EBM delivered to the right or wrong baby.
Quality of evidence	Very low
Study 3.	
Study	Davanzo R, De Cunto A, Travan L, et al. 2013. To feed or not to feed? Case presentation and best practice guidance for human milk feeding and Group B streptococcus (GBS) in developed countries.[17]
NHMRC Level of Evidence	IV Case report
Objectives	To determine the role that infected GBS positive breast milk may play in being a potential source of GBS reinfection.
Population	Preterm infants
Methods	Case study in hospital setting
Main results relevant to question	Group B streptococcus is the most frequent cause of neonatal sepsis and meningitis. Most cases occur in first week of life, and are related to vaginal carriage in the mother (early-onset disease). Late-onset disease is less common, usually transmitted via maternal hand, hospital staff or via nosocomial pathways. Moreover, 0.5% to 3% of infants with early or late-onset GBS infections will suffer from a recurrence and will often have a history of prematurity. Recurrences may be multifactorial, but are often due to persistent colonisation of the infant's oropharynx, and/or infected maternal breast milk.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to	Recurrent GBS infection after effective treatment of the first episode may be related to repeated intake of contaminated milk.

question	
Summary of limitations	Case study report only
Reviewer summary of evidence	Uncertain/not known if results apply to EBM delivered to the right or wrong baby.
Quality of evidence	Very low
Study 4.	
Study	Landers S and Updegrove K. 2010. Bacteriological screening of donor human milk before and after Holder pasteurization.[18]
NHMRC Level of Evidence	III-3 Comparative study without concurrent controls
Objectives	To determine the bacterial contamination of donor human milk, before and after Holden pasteurisation.
Population	Samples from 303 pools and 810 individual mother's donor milk.
Methods	All frozen milk samples were thawed and aseptically placed in pools prior to pasteurisation. Individuals and pooled samples were cultured and incubated for 48 hours. Hospital setting.
Main results relevant to question	Most human milk samples grew coagulase-negative staphylococcus and Gram-negative organisms. Normal skin flora was cultured from milk samples. The bacteriologic colonisation profiles of milk from mothers delivering prematurely were similar to those of mothers delivering at term. In 93% of milk samples there was no growth on routine bacterial cultures following routine Holder pasteurisation.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to	Holder pasteurisation was an effective means to remove any detectable bacteria from samples of donor human milk.

question	
Summary of limitations	-
Reviewer summary of evidence	The clinical significance of this pattern of low-level bacterial colonization of human milk with regard to preterm infants who are fed their mother's own milk is unclear. However the benefits of feeding a preterm infant her mother's breast milk far outweigh the potential risks of exposure to bacteria in the milk.
Quality of evidence	Moderate
Study 5.	
Study	Schanler RJ, Fraley JK, Lau C, et al. 2011 Breastmilk cultures and infection in extremely premature infants.[19]
NHMRC Level of Evidence	III-3 Comparative study without concurrent controls
Objectives	Expressed breast milk is known to be colonised by microbial species. This study determined whether serial microbial cultures of expressed breast milk predict infection in premature infants.
Population	161 mothers of premature infants (born <30 weeks gestation n=209)
Methods	Expressed breast milk was collected weekly and evaluated for microbial colony counts and identification of microbial species. Both hospital and home setting.
Main results relevant to question	There were 813 milk samples cultured for microbial species (bacteria and/or yeast) and 1963 microbial isolates were reported. There were no relationships between microbial counts and maternal age, ethnicity, education, skin-to-skin contact and infant infection. Microbial species included 60% Gram positive, 39% Gram negative and 1% yeast. 4.3% of milk samples had no microbial species isolated.
Other results of interest (outside the explicit scope of the question)	-
Author	This study highlights that random milk cultures obtained early in the postpartum period or at any stage, are not predictive of infections in premature

conclusions relevant to question	infants.
Summary of limitations	Both hospital and home setting, but no differentiation reported on.
Reviewer summary of evidence	Routine breast milk cultures are not useful in clinical practice, as they do not provide sufficient data regarding bacterial counts in relation to clinical infections in premature infants.
Quality of evidence	Moderate
Study 6.	
Study	Serra V, Teves S, Lopez de Volder A, et al. 2013. Comparison of the risk of microbiological contamination between samples of breast milk obtained at home and at a healthcare facility.[20]
NHMRC Level of Evidence	III-3 cross-sectional study
Objectives	To determine differences regarding the contamination of breast milk obtained at a healthcare facility versus at home.
Population	Breast milk samples from mothers with premature infants less than or equal to 35 weeks gestation.
Methods	280 breast milk samples were collected and analyzed (140 pairs: one sample was obtained at home and the other at the healthcare facility).
Main results relevant to question	From the total of analysed samples, 139 (49.6%; 95% CI: 43.6 to 55.6) were contaminated; and contamination was significantly more frequent in the samples obtained at home than those obtained at the healthcare facility (59.6% versus 39.6%; p=0.0008; OR: 2.25; 95% CI: 1.36 to 3.7).
Other results of interest (outside the explicit scope of the question)	-
Author	Half of the breast milk samples had bacterial growth, which was more frequent in the samples obtained at home than compared with those in the

conclusions relevant to question	healthcare setting.
Summary of limitations	The design of this study did not allow for establishing the relationship between bacterial contamination of the breast milk administered and other infectious diseases.
Reviewer summary of evidence	With breast milk expression in the home setting, adequate measures to ensure correct technique should be maximised.
Quality of evidence	Low

Human Immunodeficiency Virus (HIV)

Study 7.	
Study	Horvath T, Madi B, Iuppa I, et al. 2009. Interventions for preventing late post-natal mother-to-child transmission of HIV.[21]
NHMRC Level of Evidence	I (systematic review of level II studies)
Objectives	To collate and assess the evidence regarding interventions to decrease late postnatal MTCT of HIV, and to determine the efficacy of such interventions in decreasing late postnatal MTCT of HIV, increasing overall survival, and increasing HIV-free survival.
Population	HIV-infected women, pregnant or postpartum, and their children.
Methods	Systematic review seven studies located (six randomised clinical trials and one intervention cohort study)
Main results relevant to question	Complete avoidance of breastfeeding is efficacious in preventing MTCT of HIV. If breast milk is consumed, two interventions are efficacious in preventing transmission: 1) exclusive breastfeeding; and 2) extended antiretroviral prophylaxis.
Other results of interest (outside the explicit scope of the question)	-

Author conclusions relevant to question	Complete avoidance of breastfeeding is efficacious in preventing MTCT of HIV.
Summary of limitations	-
Reviewer summary of evidence	Complete avoidance of breastfeeding is efficacious in preventing MTCT of HIV. If breast milk is consumed, two interventions are efficacious in preventing transmission: 1) exclusive breastfeeding; and 2) extended antiretroviral prophylaxis.
Quality of evidence	Very high
Study 8.	
Study	White A, Mirjahangir JF, Horvath H, et al. (2014). Antiretroviral interventions for preventing breast milk transmission of HIV.[22]
NHMRC Level of Evidence	I (systematic review of level II studies)
Objectives	To determine which antiretroviral prophylactic regimens are efficacious and safe for reducing mother-to-child transmission of HIV through breastfeeding and thereby avert child morbidity and mortality.
Population	HIV-infected women, and their children.
Methods	Systematic review including seven randomised clinical trials.
Main results relevant to question	Where affordable, feasible, acceptable, sustainable, and safe alternatives to breast milk are available, it is recommended that HIV-infected mothers do not feed their infant with their breast milk.
Other results of interest (outside the explicit scope of the question)	Antiretroviral prophylaxis, whether used by the HIV-infected mother or the HIV-exposed infant while receiving breast milk, is efficacious in preventing mother-to-child transmission of HIV.

Author conclusions relevant to question	When status of a HIV-infected woman is known, breast milk should be avoided if replacement feeding is acceptable, feasible, affordable, sustainable and safe.
Summary of limitations	How best to prevent HIV transmission through breast milk in areas where complete avoidance of breastfeeding is not acceptable, feasible, affordable, sustainable or safe (AFASS) remains an extremely important issue, but outside the scope of this project.
Reviewer summary of evidence	Avoid breastfeeding if replacement feeding is AFASS.
Quality of evidence	Very high
Study 9.	
Study	Henrick B, Nag K, Yao XD, et al. 2012. Milk matters: soluble toll-like receptor 2 (sTLR2) in breast milk significantly inhibits HIV-1 infection and inflammation.[23]
NHMRC Level of Evidence	III-2 Cohort study
Objectives	To determine the role of breast milk sTLR2 in reducing transmission rates from HIV-infected mothers to their infants.
Population	Breast milk samples from healthy HIV-uninfected women
Methods	Milk samples were self-collected into sterile tubes within the first week and at one month, three months and six months postpartum.
Main results relevant to question	sTLR2 in breast milk may provide a dual protective role for infants receiving breast milk from their HIV-infected mothers. This is provided in two ways; through immunomodulatory and anti-viral factors.
Other results of interest (outside the explicit scope of the question)	-

Author conclusions relevant to question	sTLR2 in breast milk may provide a dual protective role for infants receiving breast milk from their HIV-infected mothers. This is provided in two ways; through immunomodulatory and anti-viral factors.
Summary of limitations	Experimental limitations may be the possibility that an unknown binding partner of sTLR2 may also contribute to the reduction of pro-inflammatory production and HIV-1 inhibition
Reviewer summary of evidence	sTLR2 in breast milk may be an important factor in infant health and be beneficial in decreasing the vertical HIV-1 transmission to infants.
Quality of evidence	Very high
Study 10.	
Study	Hoque SA, Hoshino H, Anwar K, et al. 2013. Transient heating of expressed breast milk up to 65°C inactivates HIV-1 in milk: a simple, rapid, and cost-effective method to prevent postnatal transmission.[24]
NHMRC Level of Evidence	Bench research (not defined)
Objectives	To determine if a simple and rapid method of heat treatment for expressed breast milk inhibits HIV-1 transmission as well as retaining the milk's nutritional quality.
Population	Excess expressed breast milk samples provided by healthy mothers.
Methods	The milk was pooled and stored frozen at −80 °C, then different quantities of milk were heated. As soon as the temperature of milk reached 65 °C, it was transferred to a 300 mL glass beaker and kept at room temperature for cooling.
Main results relevant to question	The nutritional, immunological, and antimicrobial properties of breast milk after heating to 65 °C were examined. Some key nutritional elements in breast milk, for example, total protein, IgG, IgA, lysozyme, and vitamin B12 did not alter significantly after heating to 65 °C($P > 0.05$).
Other results of interest (outside the explicit scope	

of the question)	
Author conclusions relevant to question	In this study, it was shown that simply heating the expressed breast milk in a pan over a stove to 65 ^{o}C inactivates HIV-1 while retaining the milk's important nutrients.
Summary of limitations	The effectiveness of this heat treatment may be limited by the volume and initial temperature of the breast milk, as well as the water used during heating.
Reviewer summary of evidence	Heat-treated expressed breast milk may be a safer alternative than exclusive breastfeeding, and would not only minimise the risk of HIV-1 transmission but also curtail the cost of using antiretroviral drugs after birth.
Quality of evidence	Bench research (not defined)

Cytomegalovirus (CMV)

Study 11.	
Study	Ehlinger E, Webster E, Kang H, et al. 2011. Maternal cytomegalovirus-specific immune responses and symptomatic postnatal cytomegalovirus transmission in very low-birth-weight preterm infants.[25]
NHMRC Level of Evidence	III-2 A comparative study with concurrent controls
Objectives	To identify the maternal virologic or immunologic components for protection against symptomatic postnatal CMV infection.
Population	Mothers of full-term infants (n=53) and VLBW (<1500 g) or <32-week gestational age preterm infants (n=85).
Methods	They investigated the magnitude of CMV-specific cellular and immune responses in fresh milk samples, and blood samples of particpants. Thirty of 85 mothers of preterm infants were CMV IgG seropositive and were assessed for CMV-specific immune responses. Twelve of 53 mothers of full-term infants were CMV IgG seropositive and were used for comparison.
Main results relevant to question	Milk immunoglobulin G (IgG) was inversely correlated to milk CMV load. However, milk CMV load and CMV-specific cellular and immune responses were similar in mothers of very low birthweight (VLBW) infants with and those without symptomatic postnatal CMV infection.

Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to question	The breast milk of CMV-seropositive mothers of VLBW infants with and without symptomatic postnatal CMV infection indicate that screening milk may not be an accurate predictor of disease risk. The inverse correlation between milk IgG and CMV load may suggest that this maternal response could assist in decreasing the CMV shedding into breast milk.
Summary of limitations	Only a small number of symptomatically infected infants.
Reviewer summary of evidence	Although the majority of CMV-seropositive women shed CMV in milk, symptomatic postnatal infection of VLBW infants occurs infrequently, suggesting that virologic or immunologic factors in milk may be associated with the risk and severity of postnatal CMV infection.
Quality of evidence	Very high
Study 12.	
Study	Hayashi S, Kimura H, Oshiro M, et al. 2011. Transmission of cytomegalovirus via breast milk in extremely premature infants.[26]
NHMRC Level of Evidence	III-3 A prospective study without concurrent controls
Objectives	This study prospectively evaluated the rate of postnatal cytomegalovirus (CMV) transmission through breast milk in extremely premature infants to address the impact of CMV infection on preterm infants during lactation.
Population	25 mothers and 27 infants (two sets of twins) with birth weights <1000 g and/or gestational ages <28 weeks.
Methods	The infants were mostly fed frozen-thawed breast milk. Breast milk, serum and urine samples were collected every two weeks and screened for CMV infection using the real-time polymerase chain reaction.
Main results relevant to	All of the 21 CMV-seropositive mothers had detectable CMV DNA in their breast milk, with a peak at four to six weeks postpartum. CMV infection was confirmed in only one infant (4.3%) who displayed almost no clinical symptoms.

question	
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to question	They reported low CMV transmission rates even in extremely premature infants, and the CMV-positive infant did not develop serious symptoms.
Summary of limitations	Very small numbers in study.
Reviewer summary of evidence	In this study setting, they mostly use frozen-thawed breast milk. These results suggest that frozen-thawed breast milk reduces CMV transmission rates, but not completely eliminates the virus.
Quality of evidence	Moderate
Study 13.	
Study	Nijman J, de Vries L, Koopman-Esseboom C, et al. 2012. Postnatally acquired cytomegalovirus infection in preterm infants: a prospective study on risk factors and cranial ultrasound findings.[27]
NHMRC Level of Evidence	III-3 A prospective observational study
Objectives	The aim of this study was to assess the risk factors of postnatally acquired CMV infection among preterm infants below 32 weeks of gestational age admitted to the neonatal intensive care unit.
Population	315 infants born <32 weeks gestation
Methods	Postnatal CMV infection was diagnosed by CMV PCR on urine collected at term-equivalent age. In CMV-positive infants, congenital infection was excluded. The authors compared the clinical and demographic data, feeding pattern and cranial ultrasound results of infected and non-infected

	patients. Logistic regression analysis was performed.
Main results relevant to question	In 39 of 315 infants, the diagnosis of postnatal CMV infection was made. The majority of CMV-infected infants (33/39.85%) did not develop any symptoms of CMV infection. The most important, independent risk factors of postnatal CMV infection were non-native Dutch maternal origin (OR 9.6 (95% CI 4.3 to 21.5)) and breast milk (OR 13.2 (95% CI 1.7 to 104.5)). The risk of infection significantly increased in infants with lower gestational age (OR 0.7 (95% CI 0.5 to 0.9)).
Other results of interest (outside the explicit scope of the question)	Lenticulostriate vasculopathy (LSV) was present significantly more often in infants with CMV infection (OR 4.1 (95% CI 1.9 to 8.8)).
Author conclusions relevant to question	Postnatal CMV infection is an asymptomatic infection among preterm infants. Infants with lower gestational age are at greatest risk of postnatal CMV infection, especially when fed with fresh breast milk from their non-native Dutch mother.
Summary of limitations	There was a high rate (22%) of missing inclusions at term-equivalent age due to difficulties with urine collection in infants.
Reviewer summary of evidence	The most important independent risk factors of postnatally acquired CMV infection among preterm infants are non-native Dutch maternal origin, breast milk and low gestational age. The majority of infected infants did not develop any clinical signs or symptoms of CMV infection.
Quality of evidence	Moderate
Study 14.	
Study	Capretti M, Lanari M, Lazzarotto T, et al. 2009. Very low birth weight infants born to cytomegalovirus-seropositive mothers fed with their mother's milk: a prospective study.[28]
NHMRC Level of Evidence	III-3 A prospective longitudinal observational study
Objectives	The aim of this prospective, observational study was to further investigate the incidence, the clinical features, and the risk factors of CMV infection in very low birth weight (VLBW) infants fed with their own mother's fresh milk.

Population	80 VLBW infants and their 68 mothers.
Methods	Infants' urine and their own mother's fresh breast milk were tested for CMV by means of culture tests once a week until discharge. CMV in infected milk and urine were genotyped. The clinical course, laboratory findings, and outcome of infants infected with CMV at two years of age were reported.
Main results relevant to question	Fifty-three mothers (78%) were CMV-seropositive at birth. CMV was detected in the milk of 21 of 53 seropositive mothers (40%), and CMV was in the urine in 9 of 26 infants (35%) fed with CMV-positive milk. The same gN-genotype was found in milk and urine. Three infected infants <28 weeks gestational age had a mild sepsis-like illness. Five more infants had neutropenia, conjugated hyperbilirubinaemia, or both. Post-natal CMV infection occurred in 1 of 19 infants with a gestational age <28 weeks who were treated at birth with intravenous immunoglobulin versus 3 of 5 non-treated infants (P<.02). Symptomatic CMV infection was associated with bronchopulmonary dysplasia. No neurosensorial sequelae were found at two years of corrected age.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to question	CMV infection via fresh human milk is mild, self-limiting, and without sequelae. Very-low GA and pre-existing chronic diseases are associated with symptomatic infection.
Summary of limitations	Small number of infected infants, and number of mother-infant pairs enrolled in the study.
Reviewer summary of evidence	The potential benefits of fresh breast milk seem to outweigh the risks related to CMV transmission in most premature infants. Pasteurization of maternal milk may be considered for infants <28 weeks gestation, but the impact on the nutritional and immunological elements of breast milk remain unclear.
Quality of evidence	Moderate

Hepatitis B (HBV)

Study 15.

Study	Shi Z, Yang Y, Wang H, et al. 2011. Breastfeeding of newborns by mothers carrying Hepatitis B virus: a meta-analysis and systematic review.[29]
NHMRC Level of Evidence	I A systematic review of level II studies
Objectives	To perform a systematic review of prospective studies to confirm the role of breastfeeding in mother-to-child transmission (MTCT) of hepatitis B virus (HBV).
Population	10 included studies of clinical controlled trials (751 infants in breastfeeding group, and 873 infants in the non-breastfeeding group).
Methods	Systematic review of 10 clinical controlled trials. Data regarding HBV intrauterine infection, MTCT, maternal blood and breast milk infectiousness, infant immunoprophylaxis methods and response, and adverse events.
Main results relevant to question	*Infectiousness of breast milk:* Two CCTs studied the infectiousness of breast milk (shown by HBsAg or HBV DNA positivity) in infants who received HBIG and HBVac joint immunoprophylaxis in the breastfeeding group (64 of 131 infants) vs the non-breastfeeding group (55 of 113 infants), with the pooled OR being 0.97 (95% CI, 0.58-1.62) (P=91; with minimum heterogeneity, I^2=0%, P=0.49). In infants who received only HBVac immunoprophylaxis, 20 of 55 infants in the breastfeeding group and 37 of 79 infants in the non-breastfeeding group had HBsAg or HBV DNA positivity from breast milk, with the OR being 0.65 (95% CI, 0.32-1.31) (1 CCT, P=0.23). There was no report of adverse effects during the process of breastfeeding.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to question	This review confirmed the role of breastfeeding in mother-to-child transmission of HBV. Breastfeeding after immunoprophylaxis did not contribute to MTCT transmission of HBV. In this review, there were no differences in maternal blood or breast milk HBV infectiousness between the breastfeeding and non-breastfeeding groups.
Summary of	Although randomised controlled trials are more convincing and popular, they are not practical or ethical in the case of breastfeeding.

limitations	
Reviewer summary of evidence	Breastfeeding (or breast milk) after immunoprophylaxis did not contribute to MTCT transmission of HBV.
Quality of evidence	Very high

Hepatitis C

Study 16.	
Study	Cottrell E, Chou R, Wasson N, et al. 2013. Reducing risk for mother-to-infant transmission of Hepatitis C virus: a systematic review for the US Preventive Services Task Force.[31]
NHMRC Level of Evidence	I A systematic review of level II studies
Objectives	To evaluate effects of mode of delivery, labour management strategies, and breastfeeding practices on risk for mother-to-infant transmission of HCV.
Population	18 observational studies included in systematic review.
Methods	14 cohort studies were of relevance regarding risk for mother-to-infant transmission of HCV. A total of 2971 mother-infant pairs reported on breastfeeding risk for mother-to-infant transmission.
Main results relevant to question	The 14 studies (two good-quality, two fair-quality, and 10 poor-quality studies) found no association between breastfeeding and risk for transmission.
Other results of interest (outside the explicit scope of the question)	The 18 observational studies evaluated the association between mode of delivery, labour management strategies, or breastfeeding practices and risk for mother-to-infant HCV transmission. Fourteen studies (two good-quality, four fair-quality, and eight poor-quality studies) found no clear association between mode of delivery (vaginal versus caesarean delivery) and risk for transmission. Two studies (one good-quality and one poor-quality study) reported an association between prolonged duration of ruptured membranes and increased risk for transmission.
Author conclusions relevant to	The 14 studies found no significant association between breastfeeding and risk of transmission.

question	
Summary of limitations	Only English-language articles were included. Studies were observational, and most had important methodological short-comings, including failure to adjust for potential confounders and small sample sizes.
Reviewer summary of evidence	Avoidance of breastfeeding does not seem to be indicated for reducing transmission risk.
Quality of evidence	Very high
Study 17.	
Study	Pfaender S, Heyden J, Friesland M, et al. 2013. Inactivation of Hepatitis C virus infectivity by human breast milk.[30]
NHMRC Level of Evidence	Bench research (not defined)
Objectives	To investigate the influence of breast milk on HCV infectiousness.
Population	Human milk samples of HCV-negative women
Methods	In order to analyse the effect of human breast milk on HCV infectiousness, pre-incubation experiments were performed. To further characterize the influence of human breast milk on HCV, they analysed its effect directly on the viral particle.
Main results relevant to question	Human breast milk reduced HCV infectivity in a dose-dependent manner. This effect was species-specific because milk from various animals did not inhibit HCV infection. The observed antiviral effect was independent of the HCV genotype, not abolished on temperature treatment and not shared by milk of different species.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to	Breast milk from HCV-positive mothers is unlikely to play a major role in vertical transmission. Some studies have reported HCV RNA in breast milk, but agree that breastfeeding, as long as not from a bleeding or cracked nipple, does not increase the rate of perinatal transmission of HCV.

question	
Summary of limitations	-
Reviewer summary of evidence	Breastfeeding does not increase the risk of HCV transmission. Human breast milk reduced HCV infectivity and stability in a dose-dependent manner.
Quality of evidence	Bench research (not defined)

Human T cell Leukaemia virus type I&II (HTLV I&II)

Study 18.	
Study	Takeuchi H, Takahashi M, Norose Y, et al. 2010. Transformation of breast milk macrophages by HTLV-I: implications for HTLV-I transmission via breastfeeding.[32]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To develop experimental systems for studying the biological roles of breast milk macrophages in MTCT of HTLV-I.
Population	Early colostrum breast milk was collected from a healthy woman.
Methods	Cell experimentation: cell preparation, cell washing, cell staining.
Main results relevant to question	Breast milk of the HTVL-I-infected mothers who may have virus-infected breast milk macrophages might transmit the virions to gastrointestinal targets in the newborn baby through breastfeeding.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions	Breast milk macrophages might be an appropriate HTVL-I reservoir involved in the mother-to-child transmission via breastfeeding. It is estimated that

relevant to question	2–5% of infected individuals develop adult T cell leukaemia/lymphoma (ATL) during their lifetime after a long latent asymptomatic period.
Summary of limitations	Not reported
Reviewer summary of evidence	Screening of pregnant women and avoiding breastfeeding by those infected resulted in a reduction of the prevalence of HTVL-I (particularly in Japan).
Quality of evidence	Bench research (not defined)
Study 19.	
Study	Matsubara F, Sagara Y, Kato Y, et al. 2014. Detection of antibodies to Human T-cell leukemia virus types I and 2 in breast milk from East Asian women.[33]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To develop a screening assay for the detection of antibodies to HTLV-1/2 in breast milk from East Asian women.
Population	545 breast milk samples.
Methods	A total of 545 breast milk samples were collected for screening, using a particle agglutination assay and line immunoassay.
Main results relevant to question	Breast milk samples were examined for HTVL-I using commercially available test kits. High-performance antibody screening (such as performed on donated blood) is used to assess the status of HTLV-I infection and to prevent further transmission. In this study, screening breast milk (as opposed to blood samples) highlights the importance of facilitating simpler screening, which is also highly accurate.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions	HTLV-I infection can be transmitted from mother to baby via breast milk, with the cause of vertical transmission being infection from lymphocytes in breast milk. Pregnant women who are carriers are recommended to artificially feed their baby with infant formula, to avoid infection.

relevant to question	HTLV-II antibodies have been detected in breast milk, and have also been isolated from individuals other than leukaemia patients.
Summary of limitations	Further studies are required on the sensitivity, specificity and reliability of assays using antibodies to HTLV-1/2 in breast milk.
Reviewer summary of evidence	Prevention of milk-borne transmission is the most efficient and feasible way to reduce the disease, therefore avoidance of breastfeeding is recommended.
Quality of evidence	Bench research (not defined)

Rubella

Study 20.	
Study	Hayakawa Y, Zhou Y, Mizuguchi M, et al. 2010. Quantitative and qualitative assay of rubella IgA antibody in breast milk.[34]
NHMRC Level of Evidence	Not defined (Bench research)
Objectives	The objective of this study was to examine and quantify anti-rubella virus IgA antibody levels in colostrum and breast milk.
Population	197 paired samples of colostrum and breast milk.
Methods	Breast milk samples were collected from postpartum mothers whose anti-rubella titers were determined during pregnancy. Anti-rubella virus IgA antibodies in breast milk samples were detected by an enzyme-linked immunosorbent assay (ELISA) test.
Main results relevant to question	There was a significantly positive correlation between anti-rubella virus antibody haemagglutination inhibition (HI) titer in serum and anti-rubella virus IGA found in breast milk. The mother's immune status indicates her ability to protect her infant through breast milk.
Other results of interest (outside the explicit scope of the question)	-
Author	Breast milk is considered effective in preventing infectious disease in infants, and the results from this study extend this observation to the prevention

conclusions relevant to question	of rubella virus infection.
Summary of limitations	Limited numbers of breast milk samples available.
Reviewer summary of evidence	Breast milk has a possible benefit in relation to protecting against the transmission of rubella virus infection.
Quality of evidence	Bench research (not defined)

Review Question 2: What is the impact of storage and transport of EBM on contamination and nutritional quality of milk?

Study 21.	
Study	Akinbi H, Meinzen-Derr J, Auer C, et al. 2010. Alterations in the host defense properties of human milk following prolonged storage or pasteurization.[47]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To assess the impact of pasteurization or prolonged storage at −20 °C on the immunologic components of human milk and the capability of the different forms of human milk to support bacterial proliferation.
Population	Already frozen EBM from 20 mothers (gestational age 29.6 weeks) compared with 18 samples of freshly collected EBM (38.9 gestational weeks).
Methods	Freshly expressed EBM was stored on ice from the time of collection and processed within two hours; frozen EBM (stored at −20 °C for four weeks) specimens were thawed for four to six hours at 48 °C before processing and analysis.
Main results relevant to question	-
Other results of interest (outside the explicit scope of the question)	*Immunological components:* • Lysozymes 32% lower in the frozen EBM compared to fresh EBM. • Lactoferrin was not significantly different between frozen and fresh EBM (P=0.55). • Immunoglobulin A (sIgA) reduced by 51% in frozen EBM compared to fresh EBM. • Lactoperoxidase reduced by 66% in frozen EBM compared to fresh EBM. • Muramidase activity 50% lower in frozen EBM compared to fresh EBM. • Peroxidase activity 69% lower in frozen EBM compared to fresh EBM. *Bacterial activity:* Concentration of *S aureus* bacteria was significantly increased in fresh and frozen EBM specimens by six hours of incubation (P<0.01). Relative to fresh EBM, bacterial counts were increased 1.8- to 2.3-fold at two hours, 2.8- to 3.8-fold at four hours, and 3.6- to 4.6-fold at six hours in frozen EBM for all bacteria tested (P<0.05). Proliferation of the Gram-negative bacteria (*Paeruginosa* and *E coli*) was significantly lower in frozen milk than in pasteurized milk at two hours of

	incubation (E coli; P<0.003) and at four and six hours (*E coli* and *P aeruginosa*; P<0.0001).
Author conclusions relevant to question	The immunomodulatory proteins in human milk are reduced by frozen storage. The correlation of the degree of diminution of protein concentration and activity in the different forms of EBM to clinical outcome is unclear, although the data from the bacterial proliferation study suggest this may be of clinical relevance.
Summary of limitations	Differences reported between fresh and frozen EBM specimens should be interpreted with caution because of the differences in the demographic characteristics between the 2 sets of donors. Finite number of host defense proteins analysed.
Reviewer summary of evidence	Freezing EBM at -20 C for four weeks seems to reduce the activity of immunological components of EBM.
Quality of evidence	Bench research (not defined)
Study 22.	
Study	Bertino E, Giribaldi M, Baro C, et al. 2013 Effect of prolonged refrigeration on the lipid profile, lipase activity, and oxidative status of human milk.[48]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To evaluate the effect of prolonged refrigeration of fresh human milk on its fatty acid profile, free fatty acid content, lipase activities, and oxidative status.
Population	Three groups of mothers (4, 8 and 5), N=17
Methods	The EBM specimens were obtained and pooled in a 300 ml sterile bottle and divided into five aliquots. One aliquot (0 hour) was analysed within three hours of collection, whereas the others (24 hours, 48 hours, 72 hours, 96 hours) were stored in the refrigerator for 24, 48, 72 and 96 hours, respectively. 40 mL from each HM aliquot were stored at −80 °C until used for lipid extraction and analysis.
Main results relevant to	*Nutrition:* Prolonged refrigeration did not affect the fatty acid composition of breast milk, and preserved both its overall oxidative status and the activity of

question	HMlipolytic enzymes. In particular, bile salt-dependent lipase activity, long-chain polyunsaturated fatty acids, and medium-chain saturated fatty acid concentrations were unaffected for up to 96 hours of refrigerated storage.
Other results of interest (outside the explicit scope of the question)	*Temperature regulation:* The temperature in the upper and lower part of the refrigerator was monitored every 5 minutes, using two mini data loggers equipped with internal probes. The mean temperature recorded for the three replicate pools of the experiment was determined to be 6.8 ± 1.1 °C, with values ranging from 4.0 to 9.6 °C. Temperature measured using a thermometer at a given moment does not necessarily represent true operating conditions. This should be taken into account by both health workers and families use domestic refrigerators for EBM storage.
Author conclusions relevant to question	Prolonged refrigerator storage of fresh EBM for 96 hours maintained overall lipid composition. The limited lipolysis during storage should be ascribed to the activity of lipoprotein lipase, responsible for the decrease in pH. Infants who receive EBM stored for up to 96 hours receive basically the same supply of fatty acids and active lipases as do breastfed infants.
Summary of limitations	Collected milk was pooled. Meaning EBM from mothers was combined for the purposes of this study. This may constitute a limitation for the generalisability of the results of this paper as this practice may not be common in everyday practice in NSW, Australia. Small sample size.
Reviewer summary of evidence	Study reports upon the same study population as the Giribaldi et al. study.[50] This may potentially limit the ability to generalise the study findings.
Quality of evidence	Bench research (not defined)
Study 23.	
Study	Hososaka Y, Nukita H, Ishii Y, Onishi A, Isonishi S, Ito F. 2013 Bacteriological Safety of Human Milk Storage.[49]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To examine the effects of different storage methods on bacterial counts and species in EBM.
Population	20 mothers

Methods	Expressed breast milk samples stored frozen at −20 °C for 4 weeks (one group thawed with running water and refrigerated for 24 hours at 5 °C another thawed in refrigerator for 24 hours), refrigerated for 24 or 48 hours at 5 °C or left at room temperature for three or six hours at approximately 20 °C. Samples analysed for bacterial counts (colony forming units [CFU]) and species and compared with fresh samples. Samples were cultured on blood agar medium and were aerobically incubated at 35 °C for 24 hours.
Main results relevant to question	*Contamination:* Bacterial growth was observed in all EBM samples as follows: • Fresh EBM: mean $7.2x10^3$ CFU/mL • Room temperature EBM (3 hours): mean $9.0x10^3$ CFU/mL • Room temperature EBM (6 hours): mean $1.0x10^4$ CFU/mL • Refrigerated EBM: mean $5.0x10^3$ CFU/mL • Frozen EBM: mean $5.3x10^3$ CFU/mL • Thawed frozen EBM: mean $4.2x10^3$ CFU/mL Summarised; frozen EBM had significantly fewer bacteria than fresh EBM samples. Samples refrigerated for 48 hours had significantly fewer bacteria than samples refrigerated for 24 hours. Samples at room temperature for three hours had significantly more bacteria than fresh samples. Bacteria in frozen samples decreased in number (10^3 to 10^2 CFUmL or 10^4 to 10^3 CFU/mL). *Bacterial species:* Each fresh EBM sample contained one to five bacterial species. Coagulase-negative *staphylococci* (CNS) were most prevalent and detected in all samples. In descending order or prevalence, bacterial species were; *alpha-Streptococcus* (10 samples), *Staphylococcus aureus*, *Streptococcus species* (three samples), *Escherichia coli* (two samples), *Klebsiella pneumoniae*, and Gram-positive rods (one sample). Frozen EBM: one to four species; CNS in all samples, *alpha-Streptococcus* (five samples), *E. coli* (three samples), *Baccillus sp*., *S. aureus* (two samples), *K. pneumonia* (one sample). Frozen and thawed EBM: CNS in all samples, *alpha-Streptococcus* (three samples), *Baccillus sp*., *S. aureus*, *K. pneumonia* (one sample). Refrigerated EBM: one to four species. Species names not reported. Number of species did not differ between 24 and 28 hour refrigerated samples from all but one mother.

	Room temperature: number of species did not differ from those in fresh EBM from 11 mothers. In two samples *E. coli* and *Baccillus sp.* were detected. Pathogenicity was ruled out in all detected *E. coli* by O antigen detection.
Other results of interest (outside the explicit scope of the question)	At three hours room temperature, one sample of formula milk contained a single colony of *Bacillius sp*.
Author conclusions relevant to question	Refrigerated EBM remains safe in bacteriological terms for at least two days at 5 C. Frozen EBM remains safe for at least one month at −20 °C. Bacterial count at the time of expression seems to directly influence the bacterial count of EBM regardless of the method of storage indicating that mothers need to take care with cleaning hands and containers. The rates of bacterial count decline due to storage method in frozen and refrigerated samples is likely to not exceed individual differences between mothers' EBM. Bacterial counts in EBM refrigerated for 48 hours are lower than fresh, frozen and room temperature milk. This was potentially related to the activities of normal immune factors present in human milk that are not compromised by refrigeration and room temperature. Bacterial strains are not eliminated by any storage method. *Alpha Streptococcus* seems to decline due to freezing and refrigeration indicating that immune factors in milk inhibit contamination. *E. coli* and *K. pneumonia* are intestinal bacteria that do not normally adhere to the nipple. It was suggested that the baby or mother's fingers may have contaminated the nipple with these species prior to expression.
Summary of limitations	Authors described no limitations. Small sample.
Reviewer summary of evidence	Conclusions regarding cleaning and sterilisation not based upon experimental evidence. Did not measure immunological factors in EBM held responsible for reduction in bacterial species. Lack of detail regarding finer details of EBM storage (e.g. container type, location in freezer/fridge).
Quality of	Bench research (not defined)

evidence	
Study 24.	
Study	Takci S, Gulmez D, Yigit S, et al. 2013. Container type and actericidal activity of human milk during refrigerated storage.[41]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To compare the association between storage container type on the bactericidal activity of human milk for different durations of refrigeration (fresh, and at 24 and 48 hours).
Population	22 mothers
Methods	Samples of EBM (approximately 10 mL each) were obtained by manual (hand) expression from each mother directly into sterile Pyrex bottles or polyethylene bags. 1 mL of EBM from each container was processed immediately after arrival to the laboratory. The remaining EBM was kept in the Pyrex and polyethylene containers at 4 °C until analysis at 24 and 48 hours. The bactericidal activity of each sample was studied. A strain of *Escherichia coli* ATCC 25922 was used to determine the bactericidal effect of human milk
Main results relevant to question	*Contamination (Three studies):* Bactericidal activity was significantly reduced in milk samples stored in polyethylene bags compared to those stored in Pyrex bottles when milk samples were stored at 4 °C for 24 and 48 hours ($P < .05$).
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to question	Pyrex bottles appear to be preferable to polyethylene bags for maintaining the bactericidal activity of human milk against *Escherichia coli* during short-term refrigeration. Short-term storage of human milk in Pyrex bottles is more appropriate than polyethylene bags for preserving its bactericidal activity against *E coli*.
Summary of limitations	Small sample size. Relatively poor generalisability: Evaluates only bactericidal activity against 1 bacterial strain in a small group of samples that cannot be generalised to

	all pathogens. The effect of polypropylene bottles was not measured. While a health care professional was present to ensure reliability of collection method, risk of contamination should be noted while expressing breast milk.
Reviewer summary of evidence	Results of this study show that refrigerated storage of human milk in glass is superior to polyethylene bags with regard to the preservation of the bactericidal activity of human milk against *E coli*.
Quality of evidence	Bench research (not defined)
Study 25.	
Study	Giribaldi M, Ortoffi MF, Giuffrida MG, et al. 2013. Effect of prolonged refrigeration on the protein and microbial profile of human milk.[50]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To evaluate the effect of prolonged refrigeration of expressed human milk on its protein and microbial profile.
Population	Three groups of mothers (4, 8 and 5), N=17
Methods	Milk was pooled milk, refrigerated, aliquoted and analysed within 96 hours. The milk samples were analysed for pH, total protein content, protein profile, secretory immunoglobulin A (sIgA), available lysine and lactose contents, and bacterial profile (total aerobic bacterial count, Enterobacteriaceae, coagulase-positive Staphylococci, lactic acid bacteria).
Main results relevant to question	*Nutrition:* Prolonged refrigeration of human milk does not affect its original lactic acid bacteria content. This observation is of particular interest, considering the relevant probiotic role for the infant gut of some breast milk lactic acid bacteria. Contamination (Microflora): Overall bacterial composition remained unaffected during the 96 hours of refrigeration. Mean values for total aerobic bacteria, enterobacteriaceae and coagulase-positive staphylococci counts remained beneath recommended concentrations for acceptability of EBM in milk banks. Despite high initial contamination, microbial counts remained constant throughout refrigeration.

Other results of interest (outside the explicit scope of the question)	*Temperature control:* Continuous temperature monitoring of the refrigerator showed a mean temperature of 6.8 ± 1.1 °C, with values ranging from 4.0 to 9.6 °C. despite the thermostat being set at 5 C. This observation is in accordance with the findings of a survey conducted on 119 domestic refrigerators, which reported that 80% of surveyed refrigerators, with nominal internal temperature set at 4 C, had a temperature of above 5 °C, while 61% were above 6 °C. The survey results combined with the findings of this survey indicate that temperature measured by thermometer at a given moment, often in combination with the number of daily or cumulative refrigerator openings, will not necessarily represent true refrigerator operating conditions. This should be taken into account by health workers and families who use domestic refrigerators for EBM storage.
Author conclusions relevant to question	Refrigeration of fresh human milk in controlled conditions for 96 hours maintained its bioactivity and nutritional quality, without compromising its microbial quality.
Summary of limitations	Collected milk was pooled. Meaning EBM from mothers was combined for the purposes of this study. This may constitute a limitation for the generalisability of the results of this paper as this practice may not be common in everyday practice in NSW Australia. Small sample size.
Reviewer summary of evidence	The main microbial communities of human milk were maintained within acceptable limits throughout 96 hours of refrigerated storage. Important markers of milk nutritional quality, bioactive proteins, and immunological components, were preserved. Previous conclusions by other authors,[80] that fresh EBM may be stored for up to 96 hours, have been upheld by this study. This previous study was a source of evidence in the 2012 NHMRC Infant Feeding Guidelines.[12]
Quality of evidence	Bench research (not defined)
Study 26.	
Study	Lev HM, Ovental A, Mandel D, et al. 2014. Major losses of fat, carbohydrates and energy content of preterm human milk frozen at −80 °C.[42]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To test the null hypothesis that storing frozen EBM for a range of 1 to 10 week as -80 C does not affect fat, protein and energy content.

Population	Twenty mothers of pre-term infants (25-35 weeks gestation)
Methods	Sixty samples of EBM obtained and frozen at −80 °C for eight to 83 days (average 43.8 days). After thawing and homogenisation, energy and macronutrients were assessed. Prior to long term storage samples were stored in polyethylene tubes in a refrigerator at −5 °C for a maximum period of 24 hours. A small aliquot of the fresh milk was sent for immediate analysis, after homogenization. Each frozen sample was thawed by heating at 40 °C in a thermostatic bath and homogenised.
Main results relevant to question	*Nutrition:* Fat, carbohydrate and energy content was significantly reduced in thawed EBM than in fresh milk (fat, fresh vs thawed: 3.72 ±1.17 vs 3.36 ±1.19 g/100 mL, Po0.001; carbohydrates, fresh vs thawed: 5.86 ±0.71 vs 4.09 ±0.96 g/100 mL, Po0.001; energy, fresh vs thawed: 64.93 ±12.97 vs 56.63 ±16.82 kcal/100 mL, Po0.0001). Protein content remained unchanged (protein, fresh vs thawed: 1.14 ±0.36 vs 1.15 ±0.37 g/100 mL, P=0.7). Only the decline in carbohydrate content correlated significantly with freezing duration.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to question	Freezing at −80 °C significantly decreased the energy content of EBM, from both fat and carbohydrates. Since the decrease in macronutrients was much higher than previously published for EBM storage at −20 °C, these results do not support freezing EBM at −80 °C as the gold standard for long-term EBM storage. These results run contrary to those reported in the 1995 book by Jensen, discussed in Ahrabi and Schanler's 2013 literature review.[81] (Jensen RG. Handbook of milk composition. San Diego: Academic Press, Inc.; 1995.)
Summary of limitations	Freezing at −80 °C is not common in clinical settings and rather reserved for laboratory settings. Analysis of the time factor was rather 'post hoc' and needs to be dealt with caution. Small sample size.
Reviewer summary of evidence	Long term freezing of EBM at -80 C should not be recommended as the gold standard practice for the prolonged storage of EBM in terms of its impact upon nutrient content.

Quality of evidence	Bench research (not defined)
Study 27.	
Study	Janjindamai W, Thatrimontrichai A, Maneenil G, et al. 2013. Soft plastic bag instead of hard plastic container for long-term storage of breast milk.[43]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To compare the fat content and contamination of EBM before and after storage for 30 days in hard polypropylene containers (HC) and soft polyethylene bags (SB) containers.
Population	Ninety mothers with a mean gestational age at delivery of 37.9 ± 1.3 weeks. Sixteen mothers had diseases before and during pregnancy (13 gestational diabetes mellitus, one overt diabetes mellitus and two HBV carriers). The study was performed in Thailand.
Methods	A total of 90 specimens of EBM were collected in HC and separated into two HC and two SB. Breasts were cleaned with sterile water before expression and a hospital-grade, automatic cyclic electric double pump was used on one or both breasts for expression. The fat content of each specimen of EBM in HC and SB was measured and cultures were performed. The specimens in the second HC and SB containers were kept frozen at -20 °C for 30 days before thawing in a water bath at 37 °C for 30 minutes and then measuring the fat content and performing bacterial cultures. The culture was eliminated if the results revealed normal skin flora (such as *Staphylococcus epidermidis* or *Streptococcus viridans*) or a number of organisms (colony forming unit, cfu) less than 105 since this may be considered a contamination.
Main results relevant to question	*Nutrition:* The means ± SD of the fat content of fresh and thawed EBM in HC were 2.98 ±0.97 and 2.66 ±0.88 g/100 mL, respectively, with a loss of 0.32 g/100 mL ($p<0.001$). The means ±SD of the fat content of fresh and thawed EBM in SB were 3.06 ±1.00 and 2.77 ±0.91 g/100 mL, respectively, with a mean loss of 0.29 g/100 mL during storage ($p<0.001$). The loss of fat content during frozen storage did not differ significantlybetween the two types of containers ($p=0.53$). There was significant decreases in the fat content after freezing at –20 °C for 30 days, of 0.32 ±0.45 g/100 mL (10.7%) and 0.29 ±0.45 g/100 mL (9.47%) in HC and SB respectively.

	Contamination: All bacterial cultures of fresh and thawed EBM in HC and SB showed only nonpathogenic organisms apart from that from one mother (both samples in both HC and SB contained *A. baumannii* and *K. pneumoniae*.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to question	Soft polyethylene bags can replace hard polypropylene containers for the long-term storage of frozen EBM for up to 30 days without deleterious effects on fat loss or contamination outcomes. Fat content of EBM in the present study may be impacted upon by the process of directly pumping EBM from an electronic breast pump into HCs prior to separation of the sample into a second HC or SB for fat content analysis. The lower fat contents of samples the HC might be explained by the greater adherence of fat to the polypropylene than to the polyethylene surfaces. Therefore to retain maximum fat content, it may be advisable to connect the SBs directly to the electronic breast pumps in normal practice.
Summary of limitations	Small volume of EBM in each sample container constrained by ethical considerations for using EBM of breastfeeding mothers.
Reviewer summary of evidence	In general, freezing EBM and -20 C caused significant fat loss regardless of container type. Comparisons between container type indicate that while minimal differences exist, SB can be used for EBM storage for long term storage of EBM up to 30 days at -20 C.
Quality of evidence	Bench research (not defined)
Study 28.	
Study	Sari FN, Akdag A, Dizdar EA, et al. 2012. Antioxidant capacity of fresh and stored breast milk: is -80 °C optimal temperature for freeze storage? [44]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To determine total antioxidant capacity and total oxidation status in fresh and freeze stored (at −80 °C) EBM during the stages of lactation.
Population	Forty-four healthy (four reported to have pregnancy related illness) mothers at 3, 8, and 30 days after birth.

	The study was conducted in Turkey.
Methods	Samples of colostrum, transitional and mature milk were collected by an electric breast pump from one breast. The total EBM volume collected (6 mL) was divided in two aliquot parts: 3 mL for fresh analysis conducted immediately after extraction and 3 mLfor storage at −80 °C for two months. The antioxidant status and oxidative stress of the fresh and stored EBM were assessed via determination of total antioxidant capacity and total oxidation status.
Main results relevant to question	Nutrition: Antioxidant capacity of transitional and mature milk decreased (p=0.0001, p=0.028, respectively); however, antioxidant capacity of colostrum did not change by storage at −80 °C (p>0.05). Total oxidation status showed no significant difference in fresh and stored EBM during the stages of lactation (p>0.05).
Other results of interest (outside the explicit scope of the question)	Total antioxidant capacity of fresh and stored EBM significantly decreased during the stages of lactation (p<0.0001, p=0.028, respectively).
Author conclusions relevant to question	Freeze storage of breast milk at −80 °C for two months seems not to be the optimal condition to preserve the antioxidant capacity of breast milk.
Summary of limitations	Freezing at −80 °C is not common in clinical settings and rather reserved for laboratory settings.
Reviewer summary of evidence	Long term freezing of EBM at −80 °C should not be recommended as the gold standard practice for the prolonged storage of EBM in terms of its impact upon nutrient content.
Quality of evidence	Bench research (not defined)
Study 29.	
Study	Rollo DE, Radmacher PG, Turcu RM, et. 2014. Stability of lactoferrin in stored human milk. Journal of Perinatology.[45]

NHMRC Level of Evidence	Not defined (bench research)
Objectives	To determine the effect of low temperature storage of EBM on the concentration of lactoferrin.
Population	Twenty samples (number of mothers not specified).
Methods	During week two to four postpartum, EBM samples were collected by mechanical pump and stored for different periods of time and at different temperatures per Centers for Disease Control and Prevention recommendations. Lactoferrin concentrations in EBM following freezing were compared with that in fresh human milk (5 to 10 mL aliquots) stored in a refrigerator at 4 to 8 °C and tested within 12 hours of collection. Five-day refrigerated specimens were analysed at 120 ±12 hours. Samples were then divided for long-term storage: in a frost-free refrigerator freezer at −18 °C for three months (similar to a home refrigerator/freezer), in a deep freezer at −20 °C for three and six months. Frozen samples were analysed at the designated intervals (±2 days). End points were selected based on storage recommendations from the Centers for Disease Control and Prevention document on Proper Handling and Storage of Human Milk.
Main results relevant to question	-
Other results of interest (outside the explicit scope of the question)	*Immunological Components:* Lactoferrin concentrations in refrigerated EBM samples were stable for five days in comparison to fresh EBM (255 ±105 mg/dL, P=0.338). After three months, lactoferrin concentrations were significantly lower at −18 °C (P=0.411) to −20 °C (P<0.001). The average decrease was 37%. Following storage for six months at −20 °C, lactoferrin decreased to 46%. The largest loss occurs due to initial freezing, although there continues to be degradation through a full six months.
Author conclusions relevant to question	Five-day refrigeration of EBM does not appreciably decrease lactoferrin levels. Freezing EBM for three months or more significantly lowers lactoferrin levels.
Summary of limitations	Small sample size.

Reviewer summary of evidence	Compared with freshly collected EBM, significant nutritional (lactoferrin) losses were seen with increasing length of freezer storage.
Quality of evidence	Bench research (not defined)
Study 30.	
Study	Silvestre D, Miranda M, Muriach M, et al. 2010. Frozen breast milk at –20 °C and –80 °C: a longitudinal study of glutathione peroxidase activity and malondialdehyde concentration.[46]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To evaluate antioxidant activity and concentration (glutathione peroxidase (GPx) and malondialdehyde (MDA)) of EBM when stored frozen, comparing the effects of two temperatures (–20 °C and –80 °C) and different storage times (15, 30, and 60 days).
Population	Ten healthy mothers. This study was conducted in Spain.
Methods	Ten 10 mL samples collected no less than 20 days after delivery from both breasts using an electric breast pump. Samples were collected and stored in polypropylene containers. Samples were divided into nine 1 mL aliquots. One was used for immediate fresh analysis, while the others were stored frozen under the following conditions: –20 °C for 15 days, –20 °C for 30 days, –20 °C for 60 days, –80 °C for 15 days, –80 °C for 30 days, and –80 °C for 60 days. Following each period, MDA and GPx activity was determined in each aliquot.
Main results relevant to question	*Nutrition:* Freezing EBM induced changes in the concentration of MDA at both –20 °C and –80 °C. These changes increased in magnitude with the duration. After 15 and 30 days, changes were not significant. After 60 days, a clear increase in oxidation was recorded at both temperatures. For GPx, enzyme activity is seen to decrease when EBM is frozen at both –20 °C and –80 °C, although to slightly different extents. At –20 °C, GPx activity gradually decreased throughout storage. This decrease was initially abrupt, and after 15 days, the GPx activity was significantly lower than in fresh milk. From 15 days activity progressively decreased. After 60 days, the GPx activity was minimal but did not disappear entirely. At –80 C, GPx activity decreased from the start of the study, although the magnitude was not significant. No marked changes in enzyme activity were

	recorded during the first 30 days. A sudden drop in GPx activity was seen to occur in the period after 60 days. At 60 days, remaining GPx antioxidant activity was similar at both –20 °C and –80 °C. However, enzyme activity in the intermediate stages of this storage period differed at both temperatures, and during the first 15 and 30 days. Storage at –20 °C affected the enzyme activity significantly more than storage at –80 °C.
Other results of interest (outside the explicit scope of the question)	Glutathione peroxidase should be the parameter selected for evaluation of antioxidant content and activity due to its sensitivity to the effect of temperature especially early the loss of antioxidant properties. Malondialdehyde concentration may be a better parameter to evaluate changes in later stages of oxidative stress as after 60 days of freezing storage, there are significant changes both in GPx activity and MDA concentration.
Author conclusions relevant to question	Freezing EBM causes loss in antioxidant properties which increase with the duration of storage and differ in intensity according to the temperature. To maximally preserve the antioxidant properties of EBM, it is advisable to store EBM at –80 °C for no more than 30 days, as opposed to shorter time periods at the more common temperature of –20 °C.
Summary of limitations	Small sample size. Freezing at -80 °C is not common in clinical settings and rather reserved for laboratory settings.
Reviewer summary of evidence	To retaining the antioxidant properties of EBM subjected to freeze preservation before consumption, the advisable storage temperature is –80 °C, for a maximum duration of 30 days.
Quality of evidence	Bench research (not defined)
Study 31.	
Study	Marín ML, Arroyo R, Jiménez E, et al. (2009) Cold storage of human milk: Effect on its bacterial composition.[51]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To evaluate the effect of cold storage on the natural bacterial composition of breast milk.
Population	34 healthy mothers with EBM collected either by manual expression (n=27) or breast pump (n=7)

Methods	Samples were plated onto several culture media immediately after arrival at the laboratory (day zero) and after storage at -20 °C for six weeks. To assess whether there were bacterial fluctuations through the storage period, all of the samples were analyzed at week 6; additionally, 17 were also analysed at weeks two and four. All of the frozen samples were thawed at 4 °C to 6 °C before processing.
Main results relevant to question	Authors used bacterial counts to measure immunological activity
Other results of interest (outside the explicit scope of the question)	*Bacterial counts:* No statistically significant differences were observed between counts obtained at day zero and after six weeks of storage in those media in which growth was detected. In all of the culture media, bacterial counts in pump-collected samples were higher than in those obtained by manual expression. Staphylococci and streptococci were the predominant bacteria in both fresh and frozen EBM, *Staphylococcus epidermidis* were the most abundant species at both sampling times. Lactic acid bacteria and *bifidobacteria* were also present in fresh and frozen EBM, but among them, only 1 species (*Lactobacillus gasseri*) could be isolated at both sampling times.
Author conclusions relevant to question	The results of this study suggest that cold storage of EBM at -20 °C for six weeks does not significantly (no statistically significant difference) affect either the quantitative or the qualitative bacterial composition of EBM. In relation to specific bacterial species, results suggest no significant changes in the bacterial diversity between fresh EBM and EBM after cold storage for 6 weeks at -20 °C.
Summary of limitations	Tested bacterial counts to ascertain bactericidal quality of EBM – the study did not measure the impact on the active immunological components of EBM directly.
Reviewer summary of evidence	The authors also note that many milk pumps and/or their accessories cannot be sterilised and bacteria often persist after application of current cleaning protocols. Therefore, the design of new pumping devices that can be sterilised and more efficient cleaning and disinfections procedures are warranted.
Quality of evidence	Bench research (not defined)
Study 32.	
Study	Chang J C, Chen C H, Fang L J, et al. 2013. Influence of prolonged storage process, pasteurization, and heat treatment on biologically-active human milk

	proteins.[52]
NHMRC Level of Evidence	Not defined (bench research)
Objectives	To evaluate the effects of storage procedures on the bioactive components of EBM.
Population	14 healthy lactating mothers
Methods	Three forms of human milk (freshly expressed, frozen at -20 °C for a prolonged duration (at least four weeks), and pasteurised milk) were collected from milk collected for donation to a milk bank (for the frozen EBM). Frozen EBM was thawed for 24 hours and bathed in two groups at 40 °C and 60 °C for 30 minutes prior to analysis. The concentrations of major bioactive proteins (secretory immunoglobulin A, lactoferrin, lysozyme, and leptin) were quantified using enzyme-linked immunosorbent assay kits. Changes in these proteins by heat treatment at 40°C or 60 °C for 30 minutes were further evaluated.
Main results relevant to question	-
Other results of interest (outside the explicit scope of the question)	*Immunological Components:* Mean concentrations of lactoferrin in pasteurized milk and frozen milk were 66% and 11.5% lower, respectively, compared with that in fresh milk ($p<0.0001$ and $p=0.445$). Compared with fresh milk, the concentration of lysozyme in frozen milk was 39.8% lower ($p<0.0001$). The concentration of lysozyme was slightly lower in pasteurised milk, but much lower in frozen milk in comparison with that in fresh milk ($p=0.078$ and $p<0.0001$, respectively). The mean concentrations of sIgA were 8.2% and 25.9% lower in frozen milk and pasteurised milk, respectively ($p=0.171$ and $p=0.0004$), compared with that in fresh milk. In comparison with fresh milk, sIgA in frozen milk was lower but not significantly so ($p=0.171$). No differences in concentrations of leptin were found among the three forms of human milk ($p=0.153$).
Author conclusions relevant to question	Various freezing/heating/pasteurisation processes applied to human milk prior to delivery to neonates could affect the concentration of immunomodulatory proteins, especially lactoferrin, secretory immunoglobulin A, and lysozyme. Leptin was unaffected by the various handling processes tested. Fresh milk was found to be the best food for neonates. Further studies are warranted to evaluate the functional activity of these proteins and their effects on infants' immunological status.
Summary of limitations	Frozen samples of EBM were stored for "at least" four weeks with no other duration noted.

Reviewer summary of evidence	This study supports previous findings about storage of EBM at -20 °C. Lactoferrin, sIgA, and lysozyme, the most frequently studied immunoactive proteins in human milk, are susceptible to some degradation due to freezing for prolonged periods.
Quality of evidence	Bench research (not defined)

Review Question 3: What is the evidence about disinfection and cleaning procedures for handling EBM?

Study 33.	
Study	Becker G E, Cooney F, Smith H A. 2011. Methods of milk expression for lactating women.[56]
NHMRC Level of Evidence	I (systematic review of level II studies)
Objectives	To assess acceptability, effectiveness, safety, effect on composition, contamination and cost implications of methods of milk expression.
Population	632 included in analysis (10 studies)
Methods	Systematic review (1982-2011) 23 studies located Selection criteria: Randomised and quasi-randomised trials comparing methods at any time after birth, and crossover trials commencing at least 28 days after birth.
Main results relevant to question	*Contamination (Three studies):* No evidence of difference was found for milk contamination or adverse effects between hand-expressed versus pump expressed milk. (Two studies) In one 1989 study comparing breast cleansing with an antibacterial soap to washing with water descriptively reported lower staphylococcus colony counts in the breast cleansing (intervention) group (P=0.013) (n=65).
Other results of interest (outside the explicit scope of the question)	*Nutrient quality (One study):* Higher sodium concentration in hand expressed milk compared to: • Manual pump (SMD 0.59 mmol/L, 95% CI 0.22 to 0.96, P=0.002) • Electric pump (SMD 0.70 mmol/L, 95% CI 0.32 to 1.09, P=0.0003) and lower potassium concentration in hand expressed milk compared to: • Manual pump (MD -0.37 mmol/L, 95% CI 0.00 to 0.73, P=0.05) • Electric pump (SMD -0.32 mmol/L, 95% CI -0.69 to 0.06, P=0.10) *Nutrient quality:* Hand expression may be more suitable in the first few days to initiate milk supply, and particularly where the constituents of the milk may be important.

	No evidence of difference was found for energy content between hand-expressed and pump expressed milk.
Author conclusions relevant to question	*Contamination:* We found no evidence that a particular type of pump was associated with a higher level of milk contamination.
Summary of limitations	Small sample sizes, large standard deviations, small number of studies reviewed, and the diversity of the interventions argue caution in applying these results beyond the specific method tested in the specific settings.
Reviewer summary of evidence	The one study reporting upon EBM contamination showed that one-time use of a breast cleansing process reduced bacterial counts in the milk sample. However, as the author points out, the feasibility of using soap and anti-bacterial agents on the breasts six to eight times a day raises concerns both for the mothers' skin and the mothers' willingness to continue this process for a length of time; there may also be concerns over residues of the anti-bacterial agent in the expressed milk.
Quality of evidence	Very high
Study 34.	
Study	Karimi M, Eslami Z, Shamsi F, et al. 2012. The Effect of educational intervention on decreasing mothers' expressed breast milk bacterial contamination whose infants are admitted to Nno-natal intensive care unit. [57]
NHMRC Level of Evidence	III – 2 (Cohort study)
Objectives	To investigate outbreak of bacterial contamination in EBM among mothers with hospitalised infants in the Neonatal intensive care unit, locate the sources of contamination and to define the effect of educational intervention upon contamination reduction.
Population	50 mothers of hospitalised infants (aged 16-38 years). The study was conducted in Iran.
Methods	Samples were extracted in a laboratory context with assistance from technician using either hand expression methods or breast pump. Milk was expressed directly into a sterile container and cultured to ascertain contamination measured by the number of bacterial colonies. Mothers were then provided with a validated, piloted educational intervention around hand washing with water and soap, daily showering and or breast washing with clean water (without soap or disinfectant), naming EBM containers, not applying joint expression instruments, containers or pumps. Mothers were also

	educated to use pre-boiled, wide-mouthed glass or containers when expressing breast milk by hand and use blunt–walled container (e.g. plastic dishes) for storage. Instructions were taught about the quality of EBM preservation, dish washing, and its accessories. A day after the education intervention cultures were again gathered from the same locations and mothers.
Main results relevant to question	*Contamination:* Overall, pre-intervention 80% of mothers had infected at least one sample. Post-intervention this was reduced to 36% of mothers. Before intervention 25.4% of samples were contaminated; however after intervention only 8.2% was contamination. The main source of contamination was milk containers and pumps. Pre-intervention, 62 EBM samples (25.4%) were contaminated. Post-intervention 20 samples (8.2%) were contaminated. The greatest bacterial contamination, pre-intervention, was related to samples derived from milk collection containers (70%); this dropped to 26% post-intervention. Samples from collection pumps demonstrated that pre-intervention 20 samples (45.5%) were contaminated. Post-intervention this was reduced to seven samples (15.9%) post-intervention. Contamination from hands was 6% pre-intervention and 0% post-intervention. Contamination from breasts was 8% pre-intervention and 0% post-intervention.
Other results of interest (outside the explicit scope of the question)	Type of organisms: • *Pseudomonas* and *E. coli* (9.8% samples pre/0.8% post) • *Pseudomas* (7.4% pre/4.9% post) • *E. coli* (6.5% pre/0.8% post) • *Klebsiella* (6.1% pre/0% post)
Author conclusions relevant to question	While the possibility of EBM contamination was relatively high, educational interventions may reduce the likelihood of contamination.
Summary of	Small sample size.

limitations	The post-test was conducted one day after the education intervention. The longer term impact of education intervention can therefore not be measured. Variable supervision of mothers. No thorough description of intervention.
Reviewer summary of evidence	Relatively poorly written study, but with commonsense results based upon a logical intervention.
Quality of evidence	High
Study 35.	
Study	Rhodes J. 2011. Evidence-based recommendations for breast pumping hygiene.[58]
NHMRC Level of Evidence	Not defined (literature review)
Objectives	To discuss the research evidence for best practices in breast pumping hygiene with a focus on the specific processes involved in milk expression by breast pump.
Population	-
Methods	Literature review
Main results relevant to question	*Context:* 1 source (Boo et al.) EBM expressed at home had higher contamination levels that EBM expressed in hospitals. *Body and hand hygiene:* Maintain short, clean natural fingernails with unchipped polish. Avoid wearing rings. Wash hands thoroughly with soap and warm water for 20-30 seconds. Two sources (the Human Milk Banking Association of North America (HMBANA) and the Academy of Breastfeeding Medicine (ABM)) recommend normal breast (body) hygiene and no additional cleaning or disinfection practices prior to milk expression.

	No evidence or specific recommendation for the use of alcohol rubs and impact on EBM collection. Inconclusive evidence regarding evidence and recommendations for the use of soap (antibacterial and non-antibacterial) due to the risk of destroying commensal bacteria and adverse impact upon bacterial resistance of infants. The literature review summarises that the use of antimicrobial soaps for hand washing might be of benefit prior to breast pumping in the hospital environment but use of antimicrobial soaps may not be necessary in the home. *Breast pump disinfection:* Pumps and pump parts can be a source of EBM contamination (two sources). The literature review also recommends that pumps, in the hospital and at home, the surface upon which cleaned pump parts are to be placed prior to drying should be disinfected with disinfecting solutions or wipes. If recommended by the solution's manufacturer, the surface should be rinsed after disinfection with clean water to prevent solution contamination of washed parts. Hands should also be washed after disinfecting pumps and surfaces to prevent breast or human milk contact with disinfectant chemicals. *One source:* Washing should occur in kitchen areas not bathrooms. Pump parts in contact with EBM (or not) should be disassembled, rinsed in cool water to remove EBM residue and proteins and thoroughly cleaned with soap (antimicrobial soap may be appropriate) and water (still or running) after use. Clean paper towels or unused cloth towels may be used. Ideally, a purpose-designated basin should be used. Patient specific bottle and nipple brushes can be used to clean parts, especially tight crevices. Sponges are discouraged and not appropriate in hospital settings because they trap microorganisms. At home, damp sponges can be microwaved for 2-4 minutes. Parts should not be placed in sinks and tap handles should be touched only with clean paper towels. Rising after washing is recommended and allowed to dry on a disinfected surface. When dry, parts should be removed from surfaces and reassembled. Clean paper or single-use cloth towel may be used to dry parts. Air drying may be appropriate in some contexts. Use of a dishwasher may be appropriate – especially those with 'heat boost' and 'air dry' options. Only tubing in contact with EBM should require cleaning – manufacturers' instructions for cleaning tubing should be followed if necessary. Parts may be additionally sanitised with boiling water or microwaving in bags.
Other results of interest (outside	-

the explicit scope of the question)	
Author conclusions relevant to question	Expressing human milk as hygienically as possible will diminish the risk of pathogenic contamination and decrease microbial growth in stored human milk, however, the process is fragile and any break in technique can lead to undesired results.
Summary of limitations	Literature review rather than systematic review may introduce bias.
Reviewer summary of evidence	As a literature review this paper is a document of expert opinion with a number of selected reliable sources from outside the EBM research field used to uphold recommendations for EBM pump disinfection and cleaning.
Quality of evidence	Low – expert opinion based literature review
Study 36.	
Study	Scott C, Bradford J, Gillespie E. 2010. Achieving best practice in the management of infant-feeding equipment.[59]
NHMRC Level of Evidence	Not defined (literature review and implementation project)
Objectives	In Australia, there is lack of uniformity in the management of infant-feeding equipment. A practice review and literature review was conducted to determine adherence to best practice in the management of equipment and changes in practice were implemented and evaluated.
Population	-
Methods	Literature search and personal contact with infection control teams. Development and implementation of a practice improvement protocol.
Main results relevant to question	*Dispute around classification of equipment:* Implementation of a change in practice was difficult because many healthcare workers disputed the classification of infant-feeding equipment as 'semi-critical'. Staff who rejected semi-critical classification, proposed a modified high-level disinfection schedule which required thermal disinfection every 24 hours and between patients. This was then modified to 24 hour thermal disinfection due to the expense of conducting thermal disinfection after each

	use. Persistence was necessary when introducing best practice into this particular specialty area, where identical equipment was used in the domestic setting and processed differently there. Healthcare workers should be aware of the relevant standards pertaining to the reprocessing of equipment which they use and comply with these standards. Healthcare facilities have a duty of care to ensure that infection transmission is minimised by adhering to best practice. Lack of understanding of infection control standards and resistance to the acknowledgement that breastfeeding equipment is medical equipment, were major contributors to the two-year delay in implementing new protocols. Education and resilience maybe required to achieve best practice in this area.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to question	Healthcare workers have a duty of care to maintain appropriate infection control principles when mothers use medical equipment to assist them with breast-feeding. Equipment that is re-used for mothers and their babies for the purpose of expressing breast milk and feeding babies must at minimum undergo high-level disinfection or sterilisation between every use.
Summary of limitations	None reported by author
Reviewer summary of evidence	Equipment that used in hospital by multiple mothers for the purpose of expressing breast milk and feeding babies should be carefully disinfected and cleaned. Protocols should be in place to guide this and staff educated in terms of the process and rationale.
Quality of evidence	Low (literature review and implementation project)
Study 37.	
Study	Engur D, Camak C B, Turkmen C M, et al. 2014. A milk pump as a pource for spreading *Acinetobacter baumannii* in a neonatal intensive care unit. [60]
NHMRC Level of Evidence	Case series (IV)
Objectives	To describe an outbreak of *A. baumannii* and the results of epidemiological investigations in a neonatal intensive care unit.
Population	

Methods	The authors describe an outbreak of *A. baumannii* and the results of epidemiological investigations in a neonatal intensive care unit. The outbreak strain was isolated from the outer surface of a breast milk pump. Successfully control of the outbreak was achieved through careful review of EBM collection process.
Main results relevant to question	*Pump contamination:* A positive culture from the pump was obtained. While the exact source of this organism was unknown, the blood culture of Patient 1 was the first time that any *Acinetobacter* species had been isolated in the neonatal intensive care unit. Because the mother of this patient was expressing milk while she was touching her child, it was speculated that this mother might have be responsible from contamination of the pump. During the outbreak, several deficiencies regarding the milk collection process were found. One of the main problems was keeping the pump within the intensive care unit because of physical limitations. After this outbreak, the intensive care unit was moved to a new hospital building in which the pump was kept in a separate room. Continuous guidance during hand washing, the pumping session, and reprocessing of the kits is critical. Mothers were supported to clean their kits effectively and taught to store and label them properly.
Other results of interest (outside the explicit scope of the question)	-
Author conclusions relevant to question	Physicians should be aware that breast milk pumps can serve as reservoirs *during A. baumannii* outbreaks. Adherence to hand hygiene, monitoring of milk collection session, proper reprocessing of the collection kits, and cleaning the equipment after each use are critical steps for preventing outbreaks in neonatal intensive care units.
Summary of limitations	No empirical evidence. Epidemiological evidence only.
Reviewer summary of evidence	Infection outbreaks can occur due to inadequate disinfection of equipment and hygienic cleaning practices around the expression and handling of EMB. Careful, well implemented processes should be followed in clinical settings.
Quality of evidence	Low

Study 38.	
Study	Serra VV, Teves S, Lopez de Volder A, et al. 2013. Comparison of the risk of microbiological contamination between samples of breast milk obtained at home and at a healthcare facility.[20]
NHMRC Level of Evidence	III-3 Cross-sectional study.
Objectives	To evaluate if there were any differences regarding the contamination of breast milk obtained at a healthcare facility versus at home.
Population	53 mothers of hospitalised newborn infants
Methods	Cross-sectional study that analysed pairs of breast milk samples (one obtained at home and the other one at a healthcare facility, the same day) from mothers of hospitalized newborn infants with a gestational age ≤35 weeks. A breast milk pump was used to obtain all the samples at home. Samples with over 105CFU/mL of mesophilic aerobic bacteria, or with the presence of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, *enterobacterias*, *Pseudomonas*, *Salmonella, fungi*, and yeast were considered contaminated.
Main results relevant to question	*Contamination:* A total of 280 breast milk samples (140 pairs) from 53 mothers were analysed; 139 samples (49.6%; 95% CI: 43.6 to 55.6) were contaminated; contamination was significantly more frequent in the samples obtained at home than in those obtained at a healthcare facility (59.6% versus 39.6%; p=0.0008; OR 2.25; 95% IC: 1.36 to 3.7).
Other results of interest (outside the explicit scope of the question)	
Author conclusions relevant to question	Half of the breast milk samples had bacterial growth, which was more frequent in the samples obtained at home than those obtained at a healthcare facility.
Summary of limitations	Analysis was exploratory. The design of the study did not allow examination of the relationship between bacterial contamination of the breast milk administered and food intolerance, necrotizing enterocolitis or other infectious diseases. This relationship can be analysed with studies designed to this end.

Reviewer summary of evidence	
Quality of evidence	Low

Review Question 4: What is the evidence about operational procedures that can minimise the risk of EBM misdelivery?

Paper	Study methods (design, sample size, setting)	Misdelivery prevention method	Relevant Study findings	NHMRC Level of Evidence	Quality of evidence
Barbas KH. 2013.[65]	Survey study of 120 participants, Boston USA	Establishment of mother's milk technicians	Survey response: 100% of respondents agreed that labelling was clear and easy to understand, while 85.12% agreed that mother's milk technicians improve safety of breast milk administration.	N/A	Very low
Gabrielski L, Lessen R . 2011.[13]	Descriptive, narrative article reporting on one hospital's experiences in Colorado, USA (participants=N/A)	Milk Lab Project and bar coding	The authors state that this project has resulted in less errors, and that only one error has occurred out of 219,544 samples since introducing bar coding.	N/A	Very low
Zeilhofer UB, Frey B, Zandee J, et al. 2009 [15]	Before and after study, 15 bed NICU, Switzerland	Critical incident monitoring and implementation of strategies based on incidents (i.e. changing labelling)	Despite introducing changes to labelling, administration errors did no reduce significantly.	III-3	Low
Drenckpohl D, Bowers L, Cooper H. 2007.[66]	Descriptive, narrative article reporting on one hospital's experiences in a 35 bed NICU Illinois, USA	Six Sigma Quality Improvement methodology, development of a new policy and education for staff	The authors state that since the introduction of the new policy there had been no incidents of milk being delivered to the wrong infant.	N/A	Very low
Zhang BB, LaFleur EA, Ballweg DD, et al. 2014.[67]	Health care failure mode and effect analysis, unclear number of participants, Minnesota USA	Potential risks and strategy identification	'(a) finding dedicated EBM preparation space, (b) developing a staffing model to support milk technicians, and (c) creating a process for the electronic medical record to track feedings at the children's hospital.' (page 35)	N/A	Very low