Is the Donor Milk Used in Your NICU Commercially Sterile?

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Vegetative cells, spores and toxins can and do survive pasteurization. Medical professionals may be unaware of the differences between commercial sterility and pasteurization and the methods used by process authorities to professionally process a wide range of food, now including human donor milk. This article is intended to guide the medical professional through the technical and legal aspects of thermal processing methods as well the scientific literature that supports the need for commercially sterile milk for fragile neonates.

The foundation for the next generation of human donor milk products is commercial sterility. My company made this decision to improve safety for preterm infants as well as the economy of scale and ease of use by adopting the same professional processing method utilized by the infant formula industry for many years to process commercially sterile preterm infant formula. The process is not new, nor is it experimental.

Since introducing Co-Op Donor milk, over 1,000 preterm infants have received the product with good results. Growth and tolerance studies are complete and data will be released shortly. This type of process has never been used for human milk only because there has never been enough volume of donor milk to make it possible. The founding of the Mother's Milk Cooperative has changed all that. Nursing mothers have voted with their membership and an unprecedented volume of qualified donor milk has been collected as a result.

The type of sterile processing utilized at Medolac Laboratories is called retort processing and is based on well established scientific evidence. The temperature used to process Co-Op Donor milk is higher than the holder method but held for a much shorter time. Along with higher temperature, pressure is utilized, allowing for a more efficient thermal treatment than temperature alone. The holder method, also known as the holding method, used for many years by the dairy industry, is used by donor milk providers and by non-profit milk banks today but does not result in a commercially sterile product.

In December 2010, the FDA Pediatric Advisory Committee convened a Working Group to "obtain a better understanding of Human Milk Banking—current practices, infectious disease risks, state regulations and mitigation strategies currently used to avoid contamination of donated milk."[1] During this full day event, representatives from the milk banking industry presented their standard operating methods.

Testimony was given by many experts including William Rodriguez, MD, PhD, Science Director, Office of Pediatric Therapeutics, Office of the Commissioner, United States Food and Drug Administration. Dr Rodriguez identified the potential areas of risk for human donor milk, which included:

- Infectious disease
- Non-infectious contaminants
- Nutrition

The infectious disease risk originates from two sources:

- Intrinsic (coming from the mother)
- Extrinsic (introduced after milk is expressed)

Thus, infectious contaminants, such as Staphylococcus aureus (SA) and Group B Streptococcus (GBS) may be present in the breast milk of the mother due to mastitis or it can become contaminated after it leaves the breast from a wide variety of sources during pumping, storing and handling at a milk bank. Published research exists to support this, but in our own laboratory, our microbiologists have confirmed it by culturing hand expressed breast milk samples from donors who persistently provide donor milk that is unfit for use due to high bacterial cultures, after ruling out potential causes of extrinsic contamination (collection kits, pooling containers and pumping environment).

Using Dr Rodriguez's testimony as a guideline along with other credible sources, the infectious disease risk of donor breast milk from both sources will be examined through the lens of thermal treatment efficiencies. Two methods of thermal treatment for human milk will be explored; pasteurization and commercial sterilization. The focus is on the ability of each thermal treatment method to remove or inactivate heat resistant vegetative cells, spores and toxins that could pose a threat to hospitalized preterm infants.

A separate aspect of this issue which will not be explored here but should be noted is the intrinsic contamination of mother's own milk and the lack of routine culturing when the mother of a baby in the NICU has clinical mastitis. Foxman and her co-investigators published a study in the Journal of Epidemiology reporting that during the 12 week period of the study, 9.5% of the women had received at least one clinical diagnosis of mastitis by their medical professional and 65% had received the diagnosis

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by phone.[2] The issue becomes more complicated when one considers the issue of subclinical mastitis that would be unlikely to be identified in a non-symptomatic mother but could result in intrinsically infected milk.

This warrants further research because of the prevalence of Staphylococcus sp. and Streptococcus sp. as primary pathogens present in postpartum mastitis, the lack of routine culturing when a lactating woman presents with clinical mastitis, the lack of preprocess culturing or toxin testing in most milk banks, and the risk of neonatal sepsis in preterm infants.

An Overview of Pasteurization and Sterilization

The best explanation of pasteurization and sterilization follows: "The heat processes devised to give different degrees of shelf life to food products are usually classified either as pasteurization or sterilization. The former is a partial treatment, in that it destroys only the more labile fraction of microbial population. The latter is a complete one, because the level of surviving organisms is lowered beyond any value detectable by usual analytical practices. The two treatments differ greatly in the size of the lethal agent (heat) applied. Pasteurization is usually done at temperatures lower than 80-100°C [176°- 212°F]. Sterilization is applied at temperatures ranging from 115°C to 145°C [239°-293°F].[3] Because of the difference in temperature between the two methods, a much shorter treatment time is possible with sterilization.

Pasteurization

Milk banking has grown tremendously in the past few years since a growing body of evidence supports its use for preterm infants in clinical settings. Brazil claims the record for the largest number of human milk banks in the world. This large milk banking system utilizes pasteurization as their thermal treatment. Numerous academic studies on the pasteurization of human milk have resulted. One such study conducted by the Department of Microbiology, Immunology, Parasitology and Pathology of Patologia Tropical Institute and Public Health at the Federal University of Goiás examined the microbiological quality of human milk from a Brazilian milk bank. The findings were troubling. "The presence of Staphylococcus spp., Streptococcus spp., yeasts and molds, and Enterobacteriaceae was verified in the raw milk samples." This was no surprise although for years, many promoted the idea that human milk was sterile in its raw form. "Staphylococcus aureus were isolated in 10 (5.2%) samples, Staphylococcus epidermidis in 28 (14.4%) samples, Streptococcus spp. in three (1.6%) samples, yeasts and molds in 43 (22.2%) and Enterobacteriaceae in 49 (25.3%) samples. In a hundred and forty four (144) samples which underwent thermal treatment Staphylococcus aureus was detected in five (3.5%) samples, Staphylococcus epidermidis in 15 (10.4%), Staphylococcus lugdenensis in two (1.4%), Streptococcus spp. in four (2.8%), yeasts and molds in 37 (25.7%), and Enterobacteriaceae in nine (6.3%)."[4]

Many milk banks now rely solely on post-pasteurization culturing to confirm the absence of vegetative cells of Staphyococcus sp. and Streptococcus sp. among other potential pathogens. In a study commissioned by Food Standards Australia and New Zealand, Juffs and Deeth named SA as one of the most common causes of food poisoning and identified the endotoxin produced by SA as the central cause of illness rather than the vegetative cells. The cautionary note from this study gives reason to explore this issue further as it relates to preterm babies. "Thus absence or low numbers of S. aureus in a heat treated food product does not guarantee its safety; absence of the enterotoxin must also be demonstrated. Species of Staphylococcus other than S. aureus can produce enterotoxins, but the overwhelming majority of staphylococcal food poisoning outbreaks have been caused by S. aureus."[5]

The reliance on pasteurization by milk banks to assure the absence of SA, combined with the lack of pre-process microbiological testing creates the potential for heat stable enterotoxins, which are the root cause of foodborne illness in processed foods, including milk. Karthikeyen et al reported on SA and its strong association with neonatal sepsis[6] and Romano-Bertrand[7] reported on a one year review of SA carrier, colonized or infected patients in neonatal care centers in which the investigators also screened isolates for genes encoding staphylococcal enterotoxins A(sea). They noted that both Coagulase negative staphylococci (CoNS) and Staphylococcus aureus (SA) are the main and often sole bacteria colonizing the digestive tract of low birth-weight infants during the 3 first weeks of life. Furthermore, CoNS and SA are responsible for most infections in hospitalized preterm infants. Holmes[8] and Delgado[9] cited SA as one of the main etiological agents of mastitis while Reddy[10] reports a growing trend of postpartum mastitis now seen in "as many as one third of breastfeeding women in the United States and leads to breast abscess formation in ≈10% of cases. Although breast milk cultures are not routine in PPM management, the growth of potentially pathogenic bacteria (such as β-hemolytic streptococci or Staphylococcus aureus) is associated with longer time to recovery and more frequent abscess formation. S. aureus is the most common bacterium isolated from such cultures, representing 37%–50% of isolates."

Other potential pathogens and their related toxins identified in the Brazilian study by Serafini that remain after pasteurization include the following:

Staphlococcus epidermis is one of the leading causes of neonatal sepsis and the ability of this organism to form biofilms make this potential pathogen of great concern.[11]

Streptococcus spp. A recent article by LeDoarea K and Kampmanna[12] addressed the somewhat paradoxical issues regarding, on one hand the protective components in human milk and on the other hand, the presence of potentially lethal pathogens. Low incidence is described in mothers of extremely preterm infants of 0.4%[13] and term infants of 0.82%. Higher incidence in raw milk ranged from 3.5%[14] to 10%[15] reported in donor breast milk." "The variety of delivery, treatment and storage methods of breast milk offers potential for GBS contamination. Human breast milk may contain 103 to 109 cfu/mL of GBS at any point, representing a reservoir of potential infection for the neonatal gut."[16] When mother's own milk was pasteurized before feeding her own preterm infant, researchers found no reduction in late onset sepsis.[17] Although this seems paradoxical, the inability of pasteurization to eradicate staphylococcus sp. in human milk may be the reason.

Yeast and Mold. Blachke-Hellmesen, et al analyzed 37,000 human milk samples over twenty one years and found the incidence per year of Candida albicans was found in breast milk between 8.5% and 5.2% of samples. 14.8% of the donors had delivered contaminated milk to the human milk.[18] Considering the frequency of donor milk contaminated with Candida

albicans, the researchers made recommendations about transporting donor milk at safe temperatures and to store at -20 degrees C until the laboratory analysis is complete to exclude samples contaminated by Candida albicans. This supports the need for pre-process microbiological screening in milk banking.

Enterobacteriaceae

Researchers used "deep pyrosequencing to examine the gut associated microbiome of extremely low birth infants during the first postnatal month with a first time determination of the eukaryote microbiota such as fungi and nematodes, including bacteria and viruses that have not been previously described."[19] The researchers concluded, "Together, these data reveal surprising eukaryotic and viral microbial diversity in ELBW enteric microbiota dominated bytypes of bacteria known to cause invasive disease in these infants." Many of these pathogens have been addressed herein, but others identified by these researchers need further investigation regarding the ability of pasteurization to remove them.

"Heat processing is done by the Holder method in HMBANA banks. This method can legally be used to pasteurize cow's milk (the primary method of pasteurization used for cow's milk is High Temperature Short Time—161.0 F for 15-20 seconds) and will kill or inactivate many infectious disease agents but neither the Holder method nor the High Temperature Short Time method is a sterilization procedure." (FDA Pediatric Advisory Committee, Working Group on Banked Milk Backgrounder.)

Commercial Sterilization

"Sterilization is a process employed to deprive microorganisms of their ability to multiply. The most reliable Sterilization process is obtained by application of Heat." Heat destruction of microorganisms is a gradual phenomenon: the longer the treatment time at lethal temperatures, the larger the number of killed microorganisms. Higher treatment temperatures result in a shorter time required to kill microorganisms and the heat induced damage to food products is decreased.[20]

The first commercially sterilized human donor milk, Co-Op Donor Milk™ was introduced to the hospital market last year in an effort to overcome significant barriers that have kept donor milk in short supply, resulting in rationing in neonatal intensive care units. These barriers include chronic shortages from the existing milk banking network, expensive shipping costs due to overnight shipping of frozen donor milk, waste due to short shelf life after thawing, and total cost. Because of these barriers, neonatal intensive care units and caregivers of babies at home suffering from significant feeding issues have difficulty securing a consistent supply or are unable to obtain donor milk for use after discharge. This is driving such a demand that many parents have turned to informal sources of donor milk, including those available online or through casual social networks. The risks of procuring raw milk in this way have been widely reported. Additionally, in neonatal intensive care units, powdered infant formula is not recommended because it is not sterile and the same should be required of donor milk because of the many opportunities for contamination. Infant formula used in neonatal units must now be commercially sterile due to the risk of infection from cronobacter sakazaki.[21]

Co-Op Donor Milk is thermally processed using retort processing which has been used for many years by food manufacturers and, more importantly, by manufacturers of commercially sterile preterm infant formula. "A retort is simply a vessel that is capable of withstanding extreme pressures. It is essentially a pressure cooker or autoclave. The objective of retorting is to produce a commercially sterile food. Commercially sterile food refers to a state where all pathogens and non-pathogens that could grow during the normal, unrefrigerated storage of the finished product have been eliminated. The reference standard: The spores of C. botulinum type A are normally the target organism, since they are the most durable form of any food-borne pathogen."[22]

Packaging plays a key role in retort processing. The development of flexible, multi-layer pouches for retort processing has opened the door to shorter processing times due to the improved heat permeability of the package. The package must have a hermetic seal which prevents contamination after thermal processing. Donor milk should be collected only after donors have been qualified through blood testing done at a centralized laboratory with results sent through a secure laboratory information management system. Donors should be tested for HIV 1 and 2, HTLV I and II, HBV, HCV, Syphilis, West Nile Virus and Chagas Disease. All milk should be tested for a wide range of pathogens, adulteration and other safety and quality markers prior to thermal treatment. Large pools of 1,000-2,000 gallons made up of 200-400 donors provide a wider range of immune factors such as human milk oligosaccharides.

Validation studies to develop commercially sterile donor milk must be performed by people with the experience, training and equipment to do them properly, or what is known as a process authority. Commercially sterile products fall under FDA's low acid food regulations, 21 CFR 113.

Packaging Commercially Sterile Donor Milk

Co-op Donor Milk product is packed in a flexible, retortable pouch.[23] Our retort pouch is made from several layers. The pouch is BPA free and the material that has contact with the milk is approved for such use.

According to the Codex Alimentarius Commission, commercial sterility of thermally processed food means the condition achieved by application of heat, sufficient, alone or in combination with other appropriate treatments, to render the food free from microorganisms capable of growing in the food at normal non-refrigerated conditions at which the food is likely to be held during distribution and storage. The Codex Alimentarius also calls for the exclusive use of commercially sterile, liquid feeds with premature, immune compromised infants in clinical settings, because of risk of bacterial contamination when using non-sterile feeds.[24]

The data presented here is not intended to clinically prove that there is any new risk with respect to most milk banks currently supplying hospital neonatal units. Considering the rapid increase in the use of donor milk, it is offered to encourage critical thinking and analytical review by neonatal departments charged with the wellbeing of preterm infants.

There are more questions than answers. Some need to be reviewed internally by neonatal intensive care units. Do mothers with clinical or subclinical mastitis produce milk that could put their preterm infant at risk due to high colony counts of pathogens such as S. aureus? Does the current practice of not culturing milk from nursing mothers of fragile neonates have

sufficient data to support that practice? And finally, does the current evidence support the continued use of non-sterile donor milk for preterm infants?

While it is widely accepted that the exclusive use of human milk reduces mortality and morbidity in preterm infants, hospitals should develop their own standards for assessing the quality and safety of donor milk for use when mother's milk is not available.

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