pretreatment before procedure, and the other one without EMLA cream before procedure. A time gap of more than 72 h was required between the venipunctures. The scores of the 'Neonatal Pain, Agitation and Sedation Scale' (N-PASS) [3, 4] of each enrolled premature infant were measured before, during and 10 min after venipuncture with and without EMLA cream pretreatment. The result revealed a significant difference between the N-PASS scores during venipuncture without EMLA pretreatment  $(3.2 \pm 2.3)$  and with EMLA cream pretreatment  $(1.5 \pm 1.6; \text{ paired difference: } 1.7 \pm 2.2, p = 0.000, \text{ by}$ paired *t*-tests), demonstrating that EMLA cream can effectively minimize the pain of a venipuncture. Figure 1 shows the mean N-PASS scores of the study infants without and with EMLA cream pretreatment at the three time points. To investigate the significant interaction between group (without and with EMLA pretreatment) and time points, we used repeated analysis of variance and Bonferroni multiple comparisons to examine pain levels at the three time points with and without EMLA cream pretreatment. A between-group difference was found regarding the time point of the procedure. No adverse effect was observed in this study.

The strength of this study is that repeated measurements were taken of each participating infant, and paired t-tests were used to compare the N-PASS scores without and with EMLA cream pretreatment, which differs from the majority of previous reports [5, 6]. This repeat-measures study design eliminates confounding factors, such as differentpainthresholds, among the different patients.

According to the N-PASS user guidelines, treatment or interventions for known pain and painful stimuli are suggested for scores of more than 3 points. This study revealed that themeanN-PASS score during venipuncture without EMLA cream pretreatment was3.2 points-more than 3 pointswhereas the score decreased to 1.5points when EMLA cream was applied before the procedure. These results confirm he suitability of using the N-PASS pain assessmenttool in NICUs, and that EMLA cream intervention is effective for relieving the pain of venipuncture.

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#### References

- 1. Tsou K. The morbidity rate and survival rate in extremely low birth weight premature infants in Taiwan-ten years of change. In: Tsou K (ed.). The Prognosis of Taiwanese Infants With Extremely Low Birth Weight During a Ten Year Period: Taipei, Taiwan, Premature Baby Foundation of Taiwan, 2011;5-24.
- 2. Puchalski M, Hummel P. The reality of neonatal pain. Adv Neonatal Care 2002;2:233-44;quiz 45-7.
- 3. Hummel P, Puchalski M, Creech SD, et al. Clinical reliability and validity of the N-PASS: neonatal pain, agitation and sedation scale with prolonged pain. J Perinatol 2008;28:55-60.
- 4. Hummel P, Lawlor-Klean P, Weiss MG. Validity and reliability of the N-PASS assessment tool with acute pain. J Perinatol 2010;30:474-8.
- 5. Acharya AB, Bustani PC, Phillips JD, et al. Randomised controlled trial of eutectic mixture of local anaesthetics cream for venepuncture in healthy preterm infants. Arch Dis Child Fetal Neonatal Ed 1998:78:F138-42.
- 6. Lindh V, Wiklund U, Hakansson S. Assessment of the effect of EMLA during venipuncture in the newborn by analysis of heart rate variability. Pain 2000; 86:247-54.

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How Long Does Flash-Heated Breast Milk Remain Safe for a Baby to Drink at Room Temperature?

Key words: flash heating, breast milk, HIV, Staphylococcus aureus, Escherichia coli.

### Introduction

In 'HIV and Infant Feeding' (2009), World Health Organization recommend heat-treated expressed breast milk as one option for feeding infants of human immunodeficiency virus (HIV)-positive mothers [1]. World Health Organization also recommended more research on the practicalities and feasibility of this method.

HIV in expressed breast milk can be killed, while retaining nutritional value and immune properties,

through a heat-treatment procedure called flash heating [2–4]. Flash heating represents a low-technology home-based pasteurization method for low-resource settings.

In environments where there is no refrigeration, stored flash-heated breast milk could become contaminated with bacteria that could cause infant illness. *Staphylococcus aureus* and *Escherichia coli* are two common bacteria that contaminate breast milk [5].

A previous study confirmed safety of breast milk for 8 h after flash heating [6]. In environments without refrigeration, where a mother leaves her infant and goes to work, 8 h is too short. Another study examined a 12 h interval after breast milk underwent Pretoria pasteurization, a process similar to flash heating [7], and concluded breast milk could be safely stored at room temperature for 12 h [8]. Flash heating has been shown to be superior to Pretoria pasteurization for eliminating HIV viral activity [9].

This study examines the presence of bacterial contamination in unrefrigerated flash-heated breast milk for 24 h.

#### Methods

Expressed breast milk was obtained from the Milk Matters breast milk bank (Cape Town, South Africa). The breast milk was flash heated according to procedures described by Israel-Ballard [2]. A volume of 60 ml of milk was placed into each of eight glass jars and then placed into a pot of water at room temperature. The pot of water was brought to a rolling boil. The glass jars were taken out of the boiling water and placed at room temperature. One glass jar was placed in a freezer every 4h for 24h. One glass jar of unheated breast milk was also placed in the freezer at time zero. The date, time and room temperature were noted before placing each specimen in the freezer. The temperature of the freezer was  $-22^{\circ}$ C. The glass jars of frozen breast milk were taken on ice to the South African Bureau of Standards laboratory. At South African Bureau of Standards, each container was tested for S. aureus and E. coli.

### Results

The room temperature during the study ranged from 29 to 38°C. For each 4h time interval through 24h, there was no harmful growth of either bacterium.

### Conclusion

After testing flash heated expressed breast milk, no *S. aureus* or *E. coli* colonies grew in unrefrigerated breast milk for 24 h. This is consistent with previous studies that examined bacterial contamination of heat-treated expressed breast milk for shorter time

periods [6, 8], and also with research indicating flash heating does not destroy bacteriostatic properties in breast milk [10].

This extends the period for which heat-treated expressed breast milk may be considered safe to store at room temperature. HIV-positive mothers living without access to refrigeration can be reassured that heat-treated breast milk is safe for their infant to drink for up to 24 h after flash heating.

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# References

- 1. WHO. HIV and infant feeding: revised principles and recommendations rapid advice Geneva: World Health Organization, 2009.
- Israel-Ballard K, Donovan R, Chantry C, *et al.* Flash heat inactivation of HIV-1 in human milk: a potential method to reduce postnatal transmission in developing countries. J Acquir Immune Defic Syndr 2007;45: 318–23.
- Israel-Ballard K, Abrams B, Coutsoudis A, et al. Vitamin content of breast milk from HIV-1 infected mothers before and after flash-heat treatment. J Acquir Immune Defic Syndr 2008;48:444–9.
- Chantry CJ, Israel-Ballard K, Moldoveanu Z, et al. Effect of flash-heat treatment on immunoglobulins in breast milk. J Acquir Immune Defic Syndr 2009;51: 264–7.
- Ajusi JD, Onyango FE, Mutanda LN, *et al.* Bacteriology of unheated expressed breast milk stored at room temperature. East Afr Med J 1989;66:381–7.
- Israel-Ballard K, Coutsoudis A, Chantry CJ, et al. Bacterial safety of flash-heated and unheated expressed breast milk during storage. J Trop Pediatr 2006;52: 399–405.
- Jeffrey BS, Webber L, Mokhondo KR, et al. Determination of the effectiveness of inactivation of human immunodeficiency virus by Pretoria pasteurization. J Trop Pediatr 2001;47:345–9.
- Jeffrey BS, Soma-Pillay P, Makin J, *et al.* The effect of Pretoria pasteurization on bacterial contamination of hand-expressed human breast milk. J Trop Pediatr 2003;49:240–4.
- Israel-Ballard K, Chantry C, Dewey K, et al. Viral, nutritional, and bacterial safety of flash-heated and Pretoria-pasteurized breast milk to prevent mother-tochild transmission of hiv in resource-poor countries: a pilot study. J Acquir Immune Defic Syndr 2005;40: 175–181.
- Chantry CJ, Wiedema J, Buehring G, et al. Effect of flash-heat treatment on antimicrobial activity of breastmilk. Breastfeed Med 2011;6:111–6.

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### Head Nodding Predicts Mortality in Young Hypoxaemic Papua New Guinean Children With Acute Lower Respiratory Tract Infection

In children under 5 years of age in developing countries, acute lower respiratory tract infection (ALRI) is a significant cause of mortality and morbidity [1]. In these children, hypoxaemia increases the risk of death >4-fold [2]. Consequently, many studies have attempted to correlate clinical signs of ALRI such as cyanosis, chest recession, fast breathing, grunting, nasal flaring and head nodding, with hypoxaemia [3-5]. However, none of these clinical signs has been shown to clearly distinguish hypoxaemic from non-hypoxaemic children. Though most of these signs are well documented, head nodding is a sign that has not been widely studied. Head nodding refers to head movements that are synchronous with each breath due to contractions of accessory muscles of respiration, and indicates severe respiratory distress [6].

We report head nodding as a clinical predictor of death in hypoxaemic Papua New Guinean children <5 years of age admitted to Port Moresby General Hospital with ALRI. Clinical procedures, ethical approval and other details of this report have been previously published [3].

Of the 77 children with ALRI, 20 were moderately hypoxaemic (SpO<sub>2</sub> < 90%), while 10 were severely hypoxaemic (SpO<sub>2</sub> < 85%). Case fatality rate was 5%, and all four children who died were severely hypoxaemic with oxygen saturations of 69, 71, 78 and 84%. A total of eight children had head nodding at the time of admission and of these, six were severely hypoxaemic and three (50%) died (Fisher exact test, p < 0.001). Head nodding was present in three of the four hypoxaemic children with ALRI who died. Other clinical signs predictive of death were cyanosis (Fisher exact test, p = 0.01), drowsiness (Fisher exact test, p = 0.01) and a respiratory rate of >60/min (Fisher exact test, p = 0.03).

Head nodding was the most significant clinical predictor of mortality in our study. This is consistent

with findings from a study in India that documented head nodding as a determining factor for mechanical ventilation, and a predictor of death in young children with ALRI [7]. Unlike cyanosis, which is often affected by skin pigmentation, anaemia and interobserver discrepancies, head nodding is easily recognizable without the need to undress the child. Furthermore, it can be easily taught to primary healthcare workers with limited training [6]. Head nodding is a clinical sign that is age dependent, and it is ALRI-specific, useful only in young children without co-morbidities [8]. This argument is supported by a recent ALRI study where a significant proportion of children had diarrhoea and malnutrition as co-morbidities [9]. In that study, metabolic acidosis did not influence the final outcome in those with and without head nodding, and the authors attributed this lack of association to co-morbidity.

In conclusion, this study shows that head nodding is an under-recognized ALRI-specific clinical predictor of mortality in hypoxaemic young children. Therefore, the presence of head nodding should prompt primary healthcare workers to give immediate priority to such patients with regards to oxygen administration or referral to an appropriate healthcare facility.

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## References

- 1. Williams BG, Gouws E, Boschi-Pinto C, *et al.* Estimates of world-wide distribution of child deaths from acute respiratory infections. Lancet Infect Dis 2002;2:25–32.
- Smyth A, Carty H, Hart CA. Clinical predictors of hypoxaemia in children with pneumonia. Ann Trop Paediatr 1998;18:31–40.
- Laman M, Ripa P, Vince J, Tefuarani N. Can clinical signs predict hypoxaemia in Papua New Guinean children with moderate and severe pneumonia? Ann Trop Paediatr 2005;25:23–7.
- Usen S, Webert M. Clinical signs of hypoxaemia in children with acute lower respiratory infection: indicators of oxygen therapy. Int J Tuberc Lung Dis 2001;5: 505–10.
- Duke T, Blaschke AJ, Sialis S, Bonkowsky JL. Hypoxaemia in acute respiratory and non-respiratory illnesses in neonates and children in a developing country. Arch Dis Child 2002;86:108–12.