Comparison of Macronutrient Composition of Human Breast Milk Using Two Methods of Analysis
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Aim: To assess the accuracy of the measurement of macronutrient levels in Human breast milk (HBM) using a filter-based mid-infrared (MIR) bedside analyzer.

Setting/Mechanisms: HBM is the preferred feeding for all infants but requires fortification to meet the nutritional needs of rapidly growing preterm infants. Standard fortification is routinely done for preterm babies based on estimates of macronutrient concentrations found in HBM. The nutrient density of HBM is known to vary widely within and between mothers’ milk supplies so standard fortification poses the risk of under- or over-estimating intake. Individualized fortification strategies using human milk analysis are being developed. Availability of accurate point of care determination of human milk macronutrients will allow a tailored approach to fortification and improve the care of the preterm infants by providing optimum nutrition in these fragile babies.

Methods: Mothers 1 week to 2 years post-delivery who were willing to provide at least a one-time 100 ml sample were recruited for this study. The study was approved by the MetroHealth IRB. All 100 ml samples, once fully thawed, were gently mixed by inverting and divided into 30 mL and a 70 mL aliquots. The 30 mL sample was analyzed by the Calais Milk Analyzer (Metron Instruments, Solon, Ohio) a filter-based mid-infrared (MIR) analyzer, and the 70 mL sample was shipped to Eurofins DQCI (Mounds View, MN) for reference laboratory analysis. Both methods of analysis measured macronutrient (true protein, fat, lactose) composition in g/dL. The reference laboratory tests included Kjeldahl (true protein), HPLC (lactose), and Mojonlier analysis.

Results: A total of 44 mothers were recruited yielding 51 samples. Macronutrient concentrations between the two methods correlated strongly for true protein \( R^2 = 0.82, p<0.0005 \) and fat \( R^2 = 0.99, p<0.0005 \), and moderately for lactose \( R^2 = 0.45, p<0.005 \). Bland-Altman plots for all three macronutrients demonstrate a high degree of agreement between the two methods, with the majority of differences between the two methods plotted against their means within 2 standard deviations from the mean difference.

Discussion: In comparison to reference laboratory analysis methods, we found the bedside MIR analyzer to be accurate and precise when measuring protein and fat concentrations in HBM. It is less accurate in the measurement of lactose which could possibly be due to oligosaccharides present in human milk which have a terminal lactose moiety attached to it which is not measured by HPLC. The bedside MIR Analyzer is accurate for measuring the most important macronutrient, protein, and can be used for studies involving tailored protein fortification. Other human milk analyzers are currently in the validation phase but currently none of the analyzers available are FDA approved for clinical use. The results obtained in this study add to the growing amount of evidence that MIR analyzers, provide accurate HBM composition data. Once approved by the FDA, point of care breast milk analysis using MIR analyzers can be used in the clinical setting to improve the nutritional management of preterm infants.
**Figure:** Human milk macronutrient concentration measured by the MIR analyzer and standard methods.  
Correlation between the two methods for true protein (Fig A), lactose (Fig B) and fat (Fig C).  
Bland-Altman plots displaying mean differences and limits of agreements between the two methods for true protein (Fig D), lactose (Fig E) and fat (Fig F).