

## **Protocol for Deuterium dilution technique in rural and urban babies in India**

On the measurement day, a baseline saliva sample will be obtained from each baby early in the morning just after awakening. Each subject will be administered a dose of 0.5g/kg body of deuterated water (Europa Scientific Ltd, Crewe, UK, 99.0%) followed by 25 ml of distilled water. This dose would increase the enrichment of the water pool of the tracee by about 800-900 parts per million (ppm). An aliquot of the dose will be stored for analysis of the dose of isotope administered that would be incorporated in the final calculations. Saliva samples will be collected after 3, 4 and 5 hours, corresponding to the time of equilibration. After noting the exact time of collection, the samples will be stored at -20°C in tightly sealed containers until analysis. The saliva samples will be analyzed for  $^2\text{H}$  by Fourier transformed Infra Red Spectroscopy (FTIR; Shimadzu 8300; Shimadzu, Vienna, Austria) equipped with an automatic sample shuttle and a pair of matched calcium fluoride sample cells with a 0.1 mm path length. For analysis, the pre- and post-dose samples will be simultaneously loaded into the instrument and automatically positioned in the light beam. This minimizes any interference due to the absorption of atmospheric carbon dioxide in the sample chamber. The infrared spectra will be measured in the range 2300-2800  $\text{cm}^{-1}$ . The calibration procedures will be followed as detailed elsewhere. Pool sizes for deuterium will be calculated from the dose of isotope administered and the mean of the 3, 4 and 5h enrichment of the isotope in body water. This calculation will also take into account the measurement of the enrichment of the dose. The fat free mass will be estimated using the measured TBW after assuming that the TBW resided entirely within the fat free mass, and the hydration of the fat free mass will be assumed from values measured earlier in infants. Then, body fat will be calculated as the difference between body weight and the FFM weight.

Stable Isotope Technique to Assess the Intake of Human Milk in Breastfed Infants. [http://www-pub.iaea.org/MTCD/publications/PDF/Pub1429\\_web.pdf](http://www-pub.iaea.org/MTCD/publications/PDF/Pub1429_web.pdf).