



Total Body Water



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Total Body Water Protocols

Background

Total body water has been measured by various dilution techniques for more than 70 years. The best and most frequent tracers are deuterium oxide or oxygen-18 labeled water. In small dose amounts, these stable isotope tracers are safe for all types of patients. The principle of the dilution technique is based on the belief that water is distributed in all parts of the body except body fat.

Sampling Protocol

Measurement of total body water requires only two samples, pre- and post dosing. A small sample (25 to 500 ul) of either blood, saliva or urine can be assayed. The sampling protocol is as follows:

1. Test subject should undergo an overnight fast or be fasted for 2 hours following a small breakfast.
2. Obtain a "pre" baseline plasma, saliva or urine sample before administration of isotope. It would be preferable to collect about 1 ml fluid, although smaller samples can be analyzed. If blood is collected, extract the plasma and place in a plastic test tube and freeze at -20 °C. A 1 ml aliquot of urine or saliva can be stored in a plastic test tube at -20 °C.
3. Weigh out the heavy water to the nearest 0.1 mg. A typical dose of isotope, 99.9% enriched in deuterium or 95% enriched in oxygen-18 is:

$$D (\text{gm D}_2\text{O}) = 0.15 \text{ gm/kg Body Weight}$$

$$D (\text{gm H}_2^{18}\text{O}) = 0.05 \text{ gm/kg Body Weight}$$

4. Administer the heavy water orally or intravenously (use IV grade only deuterium oxide). If the dose is given orally, rinse the cup with 50-100 ml of unlabeled water. Record the exact weight of isotope administered.
 5. Wait 3-6 hours for complete equilibration, at which time a second blood, urine or saliva sample should be collected.
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Sample Measurements

Send an aliquot (25-500 ul) of pre-dose sample, post-dose sample and undiluted isotope dose to Metabolic Solutions. Samples should be shipped frozen (preferably with dry ice) by express overnight service. The undiluted isotope dose should be shipped separately from the other samples in order to avoid contamination. Note: If several subjects use the same bottle of isotope, only one sample of the dose is required.

Deuterium and Oxygen-18 Measurements

Principle

Traditionally, deuterium and oxygen-18 measurements were performed with isotope ratio mass spectrometers. Metabolic Solutions began doing measurements with this technique in 1990. An alternative approach is gaining worldwide acceptance as a superior tool for trace deuterium and oxygen-18 measurements. Now, wavelength-scanned cavity ring-down (CRD) spectroscopy combines operational simplicity with quantitative precision. We currently use the CRD method. CRD instruments pulse in a short-duration narrow frequency laser light onto a gas sample in a flow cell. The gas sample absorbs the light. The absorbance is related to the intensity of the isotopic species. The signal is amplified by employing a mirrored cavity. Detectors monitor the absorbance with and without gaseous sample.

Dale Schoeller's group at the University of Wisconsin-Madison has validated the CRD spectroscopy technique for measuring doubly labeled water (Rapid Commun. Mass Spectrom. 25:3-8, 2011).

Instrumentation

Los Gatos Research Liquid Water Isotope Analyzer (LWIA) with automated injection system, version 2 upgrade (Los Gatos Research, Mountain View, CA)

General Technique

Plasma proteins are removed by adding approximately 5 mg zinc sulfate monohydrate to 25-50 ul plasma in a microcentrifuge tube. Samples are vortexed and spun at 8,000 rpm to precipitate proteins. The plasma protein-free supernatant are injected 6 times and the average of the last three measurements used for data analysis. Urine and saliva are analyzed without preparation. Highly enriched samples are diluted with water.

Quantitation

The CRD uses a standard curve of known enrichment levels of deuterium and oxygen-18 waters. At the completion of the run, the analyst processes the sample data by generating a standard curve using instrument software.

Reporting Results

The results of the CRD analysis are reported as a delta relative to a standard reference. The delta is measured in parts per thousand, expressed as (‰). The delta between sample and standard is defined as:

$$\text{Delta D} = [(\text{Ratio of Sample} - \text{Ratio of standard})/(\text{Ratio of standard})] \times 1000$$

The International Atomic Energy Agency (IAEA) in Vienna, Austria has recommended that all deuterium and oxygen-18 measurements be expressed relative to the Vienna Standard Mean Ocean Water (V-SMOW).

Accuracy

The IAEA standards comprising about 500 and 1000 ‰ D and about 250 and 500 ‰ ¹⁸O relative to V-SMOW were analyzed using CRD method. The values obtained are shown below:

Sample	Expected D Enrichment (‰)	95% Confidence Interval	Actual D Measurement (‰)
302A	508.4	505.5 -511.3	507.2
302B	996	987 - 1004	996.3

Sample	Expected O18 Enrichment (‰)	95% Confidence Interval	Actual O18 Measurement (‰)
304A	251.7	249.2 - 254.2	252.4 ± 0.2
304B	502.5	498.9 - 506.1	502.4 ± 0.3

Precision

The precision of the methodology for biological samples at 1000 ‰ is ± 2%. The per cent coefficient of variation is typically 0.75% daily and varies no more than 2% with international standards run throughout the year.

Quality Control

The analyses are supported by three quality control standards. Quality control charts are used to continually monitor the analytical method. Good laboratory practices (GLPs) are followed with complete documentation.

Calculation of Total Body Water

Measurements

The delta deuterium or oxygen-18 values for the pre-dose (δ_{pre}) and post-dose samples (δ_{pos}) are determined. The deuterium or oxygen-18 dose is diluted with tap water. The amount of dose diluted and water used is recorded. The deuterium or oxygen-18 content of the tap water (δ_{tap}) and diluted dose (δ_{dose}) are measured.

Total Body Water Calculations

Total body water (TBW) in moles is calculated from the dilution of the heavy isotope using the equation:

$$TBW \text{ (moles)} = \frac{WA}{18.02a} \times \frac{(\delta_{dose} - \delta_{tap})}{(\delta_{post} - \delta_{pre})}$$

where W = Amount of water (grams) used to dilute the dose, A = Amount of dose (grams) administered to subject, a = amount of dose (grams) diluted for analysis.

To convert TBW to kilograms: $TBW \text{ (kg)} = TBW \text{ (moles)} \times 18.02 / 1000 \text{ g/kg}$

It has been experimentally determined that deuterium oxide overestimates total body water by 4%. Some deuterium can bind to acidic amino acids of body protein or other non-exchangeable sites. Therefore, to correct for the non-exchange of deuterium in the body, the total body water measurement is divided by 1.04:

$$\text{Deuterium Corrected TBW (kg)} = TBW \text{ (kg)} / 1.04$$

It has been experimentally determined that oxygen-18 overestimates total body water by 1%. Some oxygen-18 can bind to acidic amino acids of body protein or other non-exchangeable sites. Therefore, to correct for the non-exchange of oxygen-18 in the body, the total body water measurement is divided by 1.01:

$$\text{Oxygen-18 Corrected TBW (kg)} = TBW \text{ (kg)} / 1.01$$
