



*A laboratory for stable isotope
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Measurement of Total Body Water, Extracellular and Intracellular Water

**Includes techniques using:
deuterium oxide, oxygen-18, and sodium bromide**

Technical Paper 915

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Deuterium Oxide Protocol

Background

Total body water has been measured by various techniques for more than 40 years. One of the most common total body water techniques is to administer a known amount of deuterium oxide to subjects. The principle of the technique is based on the belief that heavy water achieves equilibrium within 2 to 8 hours in all parts of the body except body fat.

Sampling Protocol

Measurement of total body water with deuterium oxide is easy since only two aqueous samples are required. A small sample (1 ml) of either blood, saliva or urine can be assayed. The sampling protocol using deuterium oxide 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) is:

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

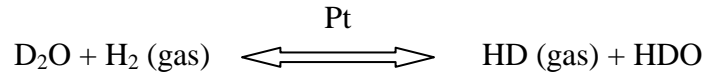
4. Administer the heavy water orally or intravenously (use IV grade only deuterium oxide). If the dose is given orally, rinse the cup with 50-100 ml of unlabeled water. Record the exact weight of isotope administered.
 5. Wait 2-8 hours for complete equilibration, at which time a second sample should be collected.
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Sample Measurements

Three deuterium measurements are necessary to determine total body water. Send an aliquot of (a) pre-dose sample, (b) post-dose sample and (c) undiluted isotope dose to Metabolic Solutions. Samples should be shipped frozen (preferably with dry ice) by express overnight service. The undiluted isotope dose must 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 Oxide Analysis

Principle



The resulting HD gas is measured with an isotope ratio mass spectrometer. The methodology is described by Scrimgeour, C.M., Rollo, M.M., Mudambo, M.K.T., Handley, L.L. and Prosser, S.J. (1993) 'A simplified method for deuterium/hydrogen isotope ratio measurements on water samples of biological origin' *Biological Mass Spectrometry*, **22**, 383-387.

Instrumentation

Europa Hydra continuous flow isotope ratio mass spectrometer, for measuring deuterium and ¹⁸O in aqueous samples following equilibration with a gas phase.

General Technique

The following steps are performed in **triplicate** for the analysis of D₂O in fluids:

Step	Action
1	0.3 ml of fluid sample is placed in a 10cc Exetainer tube.
2	0.2 ml disposable glass insert containing ~4mg of platinum on alumina powder is added to the Exetainer tube.
3	Exetainer tube is sealed and evacuated with a vacuum line.
4	Exetainer tube is filled with hydrogen gas.
5	Samples are allowed to equilibrate for seven (7) days at room temperature.
6	Samples are analyzed by IRMS within 2 weeks of the equilibration.

Deuterium Measurement

The mass spectrometer measures the sample gas against a lab reference gas (hydrogen gas generated from tap water). The ratio of mass 3 (HD) to mass 2 (H₂) is measured by the mass spectrometer. During a run, the instrument measures reference and standard gases that have been placed throughout the samples in order to assure precision and accuracy. At the completion of the run, results are drift corrected, under control of the instrument software.

Deuterium Analysis Continued

Reporting Results

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

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

The International Atomic Energy Agency (IAEA) in Vienna, Austria has recommended that all deuterium measurements be expressed relative to the Vienna Standard Mean Ocean Water (V-SMOW). Each day of analysis, a V-SMOW sample is run against the working reference gas. Results are reported relative to the working reference gas, versus V-SMOW and as atom percent excess (APE).

Correction Factors

The results of the IRMS instrument are corrected for a small contribution from H³⁺ ions generated within the mass spectrometer. The H³⁺ correction is applied with the software. The analyst minimizes the H³⁺ correction during the tuning of the instrument.

Accuracy

The IAEA standards comprising about 500 and 1000 ‰ D relative to V-SMOW were analyzed using **the hydrogen equilibration** method. The values we obtained recently are shown below:

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

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 with Deuterium

Measurements

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

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

Oxygen-18 Water Protocol

Background

Total body water measured with H₂¹⁸O is gaining acceptance as the reference method for total body water. The drawback of using an isotope of deuterium oxide is that the water space is overestimated 4% because of exchange with hydrogen of protein and other body constituents. The distribution of oxygen-18 water more accurately reflects the true volume of distribution of water than deuterium-labeled water.

Sampling

Measurement of total body water is easy with oxygen-18. The sampling protocol is as follows:

Protocol

1. Test subject should undergo an overnight fast or be fasted for 2 hours following a small breakfast.
2. Obtain a "pre" baseline plasma sample before administration of isotope. It would be preferable to collect about 1 to 2 ml serum, although smaller samples can be analyzed. Extract the serum and place in a plastic test tube and freeze.

3. Weigh out the heavy water to the nearest 0.1 mg. To calculate the correct dose of isotope (10% enriched in Oxygen-18), solve for D:

$$D \text{ (gm H}_2^{18}\text{O)} = (\text{subject wt (kg)}/70 \text{ kg})^{1/2} \times 34.58 \text{ gm}$$

34.58 gm H₂¹⁸O is the correct dose for a 70 kg adult.

4. Administer the heavy water orally or intravenously. Record the exact weight of isotope administered.
 5. Wait 2-8 hours for complete equilibration, at which time the second sample should be collected.
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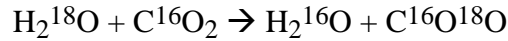
Sample Measurements

Three oxygen-18 measurements are necessary to determine total body water. Send an aliquot of (a) pre-dose sample, (b) post-dose sample and (c) undiluted isotope dose to Metabolic Solutions. Samples should be shipped frozen (preferable with dry ice) by express overnight service. Note: If several subjects use the same bottle of isotope, only one sample of the dose is required.

Oxygen-18 Analysis in Fluids

Principle

The ^{18}O content of aqueous fluid is measured by equilibration of the fluid with CO_2 of known ^{18}O content. The following reaction occurs:



The resulting CO_2 gas can be isolated and purified for isotope ratio measurement. The methodology is described by Wong, Lee and Klein, Am. J. Clin. Nutr. 45: 905-913, 1987.

Instrumentation

A Europa Scientific Tracermass isotope ratio mass spectrometer (IRMS) instrument is used for the analysis. The system uses a continuous-flow sample preparation module, Automated Breath Carbon Analyzer (ABCA). The continuous-flow preparation system converts the sample matrix into pure gas (typically CO_2 and N_2) in a helium carrier. Known reference materials are analyzed in an identical manner before and after batches of samples.

General Technique

The following steps are used to analyze ^{18}O in fluids:

Step	Action
1	Inject a known amount of reference CO_2 gas into a 10 cc Exetainer tube with a plastic syringe.
2	Inject a known amount of aqueous fluid into the Exetainer tube with a plastic syringe.
3	Equilibrate samples in a temperature controlled shaker bath for a set time period depending on the volume of aqueous fluid.
4	Prepare reference samples using identical procedure.
5	Analyze head space gas directly in Exetainer tubes with an Isotope Ratio Mass Spectrometer.

CO_2 Equilibration Time

The following table shows the amount of reference CO_2 gas added to various amounts of water sample and the equilibration time:

Aqueous Size	CO_2 Gas Added	Equilibration Time
500 μl	0.5 ml	3 hrs
250 μl	0.5 ml	4 hrs
25 μl	0.5 ml	96 hrs
10 μl	0.5 ml	96 hrs

¹⁸O Methodology Continued

Reporting Results

The results of the isotope ratio analysis are reported as a delta relative to a reference gas. The delta is measured in parts per thousand, expressed as (‰). The International Atomic Energy Agency (IAEA) in Vienna, Austria has suggested that all ¹⁸O measurements be expressed relative to the Vienna Standard Mean Ocean Water (V-SMOW). Metabolic Solutions reports results relative to the V-SMOW standard, a reference CO₂ gas and as atom percent excess (APE).

Correction Factors

There will be some isotopic fractionation between the water and the added carbon dioxide gas. However, using our water to CO₂ ratio, this fractionation error will be less than 0.01 ‰. The results are not corrected since this fractionation error is below the accuracy and precision of the method.

Accuracy

The IAEA standards comprising about 250 and 500 ‰ ¹⁸O relative to V-SMOW were analyzed using the water/CO₂ equilibration method. The values we obtained recently are shown below:

Sample	Expected Enrichment (‰)	95% Confidence Interval	Actual 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 water samples at natural abundance is ± 0.10 ‰ and for biological samples at 250 ‰ the precision is ± 0.20 ‰.

Quality Control

Two or three working standards are run daily to insure quality assurance of the analyses. Quality control charts are used to evaluate the analytical method daily. Good laboratory practices (GLPs) are followed with complete documentation.

Calculation of Total Body Water with Oxygen-18

Measurements

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

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

Extracellular Water Measurements with Sodium Bromide

Physiological Significance of Body Water

Body composition changes can often reveal inadequacy of nutritional support and the presence or progression of disease. Total body water (TBW) is used traditionally to determine body composition. TBW is composed of two independently varying compartments, intracellular water (ICW) and extracellular water (ECW). ICW consists of the fluid phase of cells, mostly in organs and muscle. ECW includes plasma, interstitial fluid, and connective tissue fluids. TBW and ECW can be measured directly from dilution of isotopes of water and bromide whereas ICW is determined indirectly by subtracting ECW from TBW.

Along with being a component of body composition, ECW is a sensitive indicator of nutritional and health status. Changes in ECW can reveal malnutrition, heart disease, and body changes due to growth (1, 2, 3). ECW volume may be decreased by dehydration or increased by edema with malnutrition (2). Monitoring ECW can greatly increase the effectiveness of clinical evaluation. For example, patients may maintain a stable body weight despite changes in body water compartments resulting from inadequate nutrition or disease. Further, repeat measurements can monitor response to treatment or effectiveness of procedures.

Measurement of ECW

ECW is defined by the volume distribution of chloride, corrected for intracellular chloride. When bromide is administered to a human or animal, it exchanges rapidly with nearly all the body chloride. The volume distribution of bromide is essentially the same as that of chloride and can be used to estimate ECW (1).

Requirements for a substance that estimates ECW include rapid equilibration in the ECW, slow excretion, and minimal penetration into tissues. One shortcoming for many components that estimate ECW is that excretion of the substance may occur before equilibrium in extracellular fluids takes place. During the time frame of the measurement, bromide losses through excretion are small and largely insignificant (4). Bromide can be used to measure ECW but will also include the volume of red cells and glandular cells and will, therefore, slightly overestimate ECW. The corrected bromide space (CBS) adjusts results for the overestimation of ECW. With this slight adjustment, the CBS is one of the most accurate and widely used methods for estimating ECW.

ECW is determined from corrected bromide space (CBS) using the following formula

$$\text{CBS} = ([\text{Br}^-] \text{ dose} / [\text{Br}^-] \text{ plasma}) * 0.90 * 0.95 * 0.94$$

where 0.90 is the correction for non-extracellular distribution, 0.95 is the Donnan equilibration factor, and 0.94 is the proportion of water in plasma. Bromide loss in urine is typically 1.6% and has a negligible effect on the measurement of CBS.

Advantages of Sodium Bromide to Measure ECW

Advantages of bromide include the ability to administer orally, good absorption, slow excretion, and only moderate penetration into tissues. Unlike most methods that employ radiolabels or require expensive instrumentation, bromide offers a safe, inexpensive, and accurate means to determine ECW. Estimates of ECW using bromide dilution space lends itself to determinations in infants, field studies, and applications involving patients receiving critical care.

Applications of the Sodium Bromide Technique

Monitoring nutritional status

Effective nutritional intervention requires sensitive methods to monitor outcomes of nutritional therapy. Most methods are insensitive, nonspecific, and do not measure body composition. Further, few methods are applicable to patients receiving critical care. Szeluga et al. addressed these issues for patients receiving bone marrow transplants and receiving nutritional support (3). Body composition was monitored serially through measurements of TBW using labeled water and ECW using bromide dilution. They concluded that serial measurements of body composition are an effective way to monitor the outcome of nutritional support.

Measurements of Body Composition

Growth hormone deficient (GHD) adults have increased body fat and decreased fat free mass. Previous determinations of TBW and ECW were based on indirect methods and produced contradictory results. Snel et al. sought to determine body composition in GHD adults by measuring ECW directly using bromide dilution (5). They found hydration state and water distribution do not differ for GHD patients and healthy controls. This finding is clinically relevant because most patients have signs and symptoms of excessive water retention during the first few months of GH-replacement therapy.

Monitoring Disease and Nutritional States of Infants

Infants are highly susceptible to stress that causes change in ECW. In the infant, the absolute extracellular volume is low compared with normal physiologic needs. As a result, the infant is frequently unable to maintain homeostasis when subjected to stress. Further, the pediatric population is at particular risk for disturbance in volume due to the high incidence of diarrhea, malnutrition, and congenital defects (2).

Measurement of ECW can help elucidate change in body composition due to disease. Cheek et al. noted that hypotonic expansion of ECW occurs with loss of water in tuberculous conditions of a generalized type or in tuberculous meningitis (2). In this case, measurement of plasma sodium concentrations alone would suggest a loss of sodium. Due to the expansion of ECW, however, plasma sodium concentration is low but may be present without loss of sodium. Thus, ECW measurements may be useful for diagnosis.

Fink and Cheek measured extracellular volume of normal newborns during the first day of life (6). They sought to determine baseline values for ECW in newborns to be used in the identification of

abnormal states of hydration. Typical abnormal states occur for the infant born of the diabetic mother, where edema is prevalent. They also reported that ECW and TBW are increased in premature infants with respiratory distress.

Observations suggest body water compartments are altered for infants born via cesarean section. Cassidy determined body water spaces for infants delivered vaginally and by cesarean section using antipyrine space to estimate TBW and bromide space to estimate ECW (7). Neonates delivered by cesarean section had a significantly increased ICW volume compared with their controls. Variation in volume and distribution of body water can influence measurements serum protein, sodium concentration, and arterial hematocrits.

ECW Provides a Clear Picture for Electrolyte Balance

An important application is the influence that ECW has on measured electrolyte concentrations. A decrease in the concentration of sodium can result from either loss of sodium or an increase in ECW. Clear assessment of electrolyte loss cannot be made without information on ECW (2).

Protocol for Estimating ECW Using Sodium Bromide

Step 1) Draw 0.5-1ml baseline blood sample.

Step 2) Administer 30-60 mg/kg sodium bromide as a 5% solution orally, intravenously, or subcutaneously.

Step 3) Wait for equilibration. Equilibration requires 2 hours for IV, 3-4 hours for oral, and 24 hours for subcutaneous administration.

Step 4) After equilibration draw second 0.5-1ml blood sample.

Step 5) Ship samples to MSI via an overnight service packaged with dry ice.

Repeat measurements can be performed after 3-4 days.

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