
A Simplified Procedure for the Measurement of Urine Iodide Levels by the Ion-Selective Electrode Assay in a Clinical Setting

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Introduction

Various procedures are available for the measurement of inorganic iodide in urine samples.^{1-3 and *vide infra*} Among these procedures, the ion-selective electrode assay is the most appropriate for a physician's office. Quantification of iodide is performed by a potentiometric method, using an iodide selective electrode (ISE). Urinary iodide is measured by the electromotive force (EMF) generated on the iodide-selective electrode due to the presence of iodide in the urine sample. Within a certain range of iodide concentrations, there is a linear relationship between the logarithm of iodide concentration and the EMF generated. The only reagents, water and sodium nitrate, used in this procedure are non-toxic and well suited for a clinical setting. Sodium nitrate (NaNO₃) is used as an Isotonic Strength Adjuster (ISA) at an initial concentration of 5 Moles/L to improve performance of the electrode. The ISA is diluted one part ISA to two parts of iodide standards and urine samples. The final concentration of NaNO₃ in the assay is 1.66 Molar.³

Although the iodide-selective electrode is very specific for iodide (Table 1), chloride may interfere in the assay of iodide when the molar concentration of chloride in the urine is six orders of magnitude greater than that of iodide.^{4,5} Due to the relatively high iodide levels observed in the urine of mainland Japanese (10⁻⁴-10⁻⁵ Molar), the ion-selective electrode can measure iodide directly in their urine samples without prior purification.^{6,7} In the US population, urinary chloride levels are usually between 100 and 300 millimolar, whereas urinary iodide levels are in the low micromolar range (10⁻⁶-10⁻⁷M). Urine iodide levels in the US population are 100-fold lower than observed in mainland Japanese.³

In order to measure the low levels of urinary iodide in the US population by the ISE method, a chromatographic procedure using strong anion exchange cartridges in a positive displacement manifold (PDM) was developed by the senior author with the assistance of a precision machinist in order to separate iodide from

the other halides — chloride, fluoride, and bromide.^{8,9} The PDM allowed semiautomation of the ISE procedure. Two models were constructed for use with 20 cartridges (PDM-20) and 40 cartridges (PDM-40). The PDM-20 is shown in Figure 1. We are now making available a PDM-6 with a small footprint for six cartridges. The PDM-6 is intended for use in a clinical set-up. The chromatographic separation of the four halides allows the combined measurement of each halide in the same sample of urine, serum, and saliva⁹⁻¹¹ (Figure 2). These cartridges have a high power of resolution with complete separation of the four halides (Table 2). Serial measurements of serum iodide, fluoride, and bromide were performed in six normal female subjects following ingestion of 50 mg iodine/iodide (Iodoral[®]). The results are displayed in Figures 3-5. The serum iodide levels in these subjects were previously reported.⁸

An iodine/iodide loading test to assess whole body sufficiency for the essential element iodine was recently developed.^{5,12} The test consists of ingesting four tablets of a solid dosage form of Lugol (Iodoral[®]), containing a total of 50 mg iodine/iodide. Then urinary iodide levels are measured in the following 24-hour collection. The iodine/iodide loading test is based on the concept that the normally functioning human body has a mechanism to retain ingested iodine until whole body sufficiency for iodine is achieved. The daily amount of iodine required for whole body sufficiency, called orthoiodosupplementation,^{3,12} ranged from 12.5-50 mg iodine.⁵

During orthoiodosupplementation, a negative feedback mechanism is triggered that progressively adjusts the excretion of iodine to balance the intake. As the body iodine content increases, the percentage of the iodine load retained decreases with a concomitant increase in the amount of iodide excreted in the 24-hour urine collection. When whole body sufficiency for iodine is achieved, the absorbed iodine/iodide is quantitatively excreted in the urine as iodide, and the adult body retained approximately 1.5 g iodine. A good correlation was observed between the results of the loading test and the clinical response to iodine intervention in these patients.¹²⁻¹⁴

For the iodine/iodide loading test to be widely used in medical practice, the measurement of urine iodide levels would be best performed *in situ*, that is, in the physician's office, using a simple procedure with non-hazardous reagents. The ISE procedure is ideal for a clinical setting, requiring only water and sodium

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chromatographic system previously described⁸ was modified and simplified to eliminate the collection of four separate fractions from the chromatographic column. Although the separation of the four halides from each other allowed their measurement in the same biological fluid,¹¹ the measurement of urine iodide levels pre-

Table 1
Interferences in the ISE Assay of Halides and Procedures for Preventing These Interferences

Halides	Interferences*	Procedures for Preventing Interference
Chloride	OH ⁻ = 80 Br ⁻ = 3 x 10 ⁻³ I ⁻ = 5x10 ⁻⁷	1) Acidification 2) Chromatographic separation from other halides
Fluoride	OH ⁻	Acidification with Orion special ISA: TSIAB, added to urine samples at equal volumes
Bromide	OH ⁻ = 3 x 10 ⁴ Cl ⁻ = 400 I ⁻ = 2 x 10 ⁻⁴	Chromatographic separation from other halides
Iodide	Cl ⁻ = 10 ⁶ Br ⁻ = 5 x 10 ³	Chromatographic separation from other halides

* The maximum allowable concentrations of interfering substances express as the molar ratio of the interfering ion concentration to the sample halide concentration. If the ratio is exceeded, the data generated by the ion-selective electrode will become unreliable. Information supplied by Thermo Orion Corporation. (From reference 5)

postorthiodosupplementation⁵ only require the purification of iodide from the other halides without the separation of the other halides from each other.

Theoretical Considerations

Specificity of the Ion-Selective Electrode: According to information supplied by Thermo Orion, chloride will interfere with the iodide electrode if the chloride/iodide molar ratio exceeds 1 million. For bromide, the iodide selective electrode is less specific, with interference expected when the bromide/iodide molar ratio exceeds 5,000 (Table 1).⁵ Since chloride and fluoride are eluted easily from the SAX (strong anion exchanger) resin using low ionic strength nitrate solution, bromide is the most likely halide to interfere with the capacity of the SAX resin to retain iodide and also to interfere with the measurement of iodide if bromide concentration exceeds those of iodide by three orders of magnitude.

Relative Selectivity of Halides for the SAX Cartridge: Materials used in anion exchange chromatography are composed of three components attached together and placed in a column or a cartridge: the base or backbone support, the functional group or ion exchanger, and the counter ion available for exchange. For backbone, styrene divinyl benzene (SDB) is preferred over silica gel because it is more rugged, less sensitive to pH changes, and possesses a higher capacity.¹⁵ For example, SAX columns with silica backbone are available from

Varian (Harbor City, CA) with a capacity of 0.85 m Eq/g of resin. However, with SDB backbone, Altech (Deerfield, IL) quotes a figure of 1.5 m Eq/g, a 75% greater capacity to exchange anions. This translates into the ability to process a 75% greater volume of urine for the same amount of resin, and a 75% greater capacity to retain iodide.

Strong anion exchangers are always charged at any pH. Therefore, elution of the analate of interest could be achieved by increasing the ionic

strength of the elution solvent without any acidification. Data are available for strong anion exchangers regarding the relative selectivity of halides. With fluoride as unity; chloride has a relative selectivity of 10, bromide 28, and iodide 87.¹⁵ The higher the number, the stronger the binding of the halide to the anion exchanger. The stronger the binding of the halide to the ion exchanger, the higher the ionic strength required for elution.

Using gradient elution, one would expect to desorb from the SAX column first fluoride, then chloride, followed by bromide, and last iodide. This pattern was observed with the Altech 500 mg SAX column #309750.³ However, when the 600 mg cartridges #21907 were used, chloride was eluted before fluoride (Figure 2). We have no explanation for this deviation from the expected.

By adding sodium nitrate to urine samples in concentrations high enough to prevent the binding of fluoride, chloride, and bromide to the SAX resin, but not high enough to interfere with the binding of iodide, a high degree of purity of the iodide fraction can be achieved. Under those conditions, the eluted urine sample would contain fluoride, chloride, and bromide, leaving iodide on the SAX resin for further elution with 10 ml of 5N NaNO₃.

The SAX cartridges used in the procedure contain the tetramethylammonium group as strong anion exchanger

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nium group. A cartridge with nitrate as counter ion would eliminate the addition of sodium nitrate to the urine samples, further simplifying this procedure. Unfortunately, the SAX cartridges are not commercially available with nitrate as counter ion.

Capacity of the SAX Cartridge: In the cartridge format, the largest capacity available with the strong anion exchanger is 600 mg of SAX resin, containing 900 μ Moles of quarternary amines. Since iodide is monovalent, the maximum amount of iodide that could be retained on the column is 900 μ M. Iodide has an atomic weight of 127, therefore the maximum capacity of the cartridge would be 114.3 mg of iodide. However, this capacity depends also on the amount of interfering substances present in the urine sample such as nitrate, thiocyanate, and bromide. In subjects on a typical Western diet, urine samples contain, at most, a few mg of nitrates and thiocyanates per liter of urine.¹⁶ The main source of thiocyanate in the US population is cigarette smoking.^{17,18} However, urinary bromide levels can reach several hundreds milligrams per liter of urine.^{5,9,10}

With increased levels of bromide sometimes observed following the iodine/iodide loading test,^{5,9} the capacity of the SAX resin for iodide would be expected to decrease significantly. In such cases, iodide would not be completely retained on the SAX column, resulting in underestimation of iodide levels. The problem with bromide is not just its interference with the binding of iodide to the SAX column. Bromide would also interfere with iodide measurement by the ISE. For example, with a urine sample containing the usually low iodide levels in the US population (0.02 – 0.5 mg/L), only 100 mg of bromide per liter of urine could cause an overestimation of the iodide levels by 0.02 mg/L. If bromide could be eluted with the urine in the first pass through the column, the full capacity of the column to retain iodide would be available. Also, the complete elimination of bromide from the iodide fraction would prevent its interference in the ISE measurement of iodide. This goal was achieved in the simple procedure described here.

Optimization of the Simplified 2-Step ISE Procedure

Deiodized urine was prepared by the method of Cooper and Croxson:⁴ Pooled urine samples were chromatographed on a SAX resin (Altech #211510), and the eluted urine was used in the following experiments. Although Cooper and Croxson⁴ called this processed urine “deiodized,” we have observed a complete elimination of bromide also from the eluted urine (less than 0.006 mg/L for iodide and less than 1 mg/L for

Figure 1

PDM-20



The PDM-20 is a semiautomated equipment designed for the chromatographic separation of the halides for biological fluids using the SAX 600 mg cartridge. It is capable of processing 20 samples of biological fluids in a batch. The PDM-40 can process 40 samples.

bromide), leaving iodide and bromide on the SAX resin. Therefore, the proper name would be “deiodized and debromized.”

The concentration of chloride in the processed pooled urine was 80 mM/L. Sodium chloride was added to reach a concentration of 300 mM/L. Bromide (25 mM/L) and fluoride (2 mM/L) were added to the treated urine, called the halide stock solution. To this stock solution, increasing amounts of iodide (potassium salt) were added: 0, 1 μ M, 10 μ M, 100 μ M, 1,000 μ M and 10,000 μ M iodide (0, 0.127, 1.27, 12.7, 127 and 1,270 mg).

Pilot experiments were performed with increasing concentrations of NaNO₃ in the urine samples prior to chromatography in order to pinpoint the final concentration of NaNO₃ in the urine sample that would result in the complete elution of chloride, fluoride, and bromide with the eluted urine, leaving only and

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strength of 1.66 M NaNO₃ prior to measurement by the iodide selective electrode. The same experiments were repeated with water instead of deiodized/debromized urine.

The data obtained when 100 μM of iodide (sodium salt) was added to the halide stock solution are displayed in Figure 6. Without NaNO₃ in the urine samples, bromide in the eluted urine was below the sensitivity (< 1 mg/L), and all the bromide (2,000 mg/L) was eluted with the second elution with 10 ml 5N NaNO₃. Some 25 times the theoretical maximum capacity of the cartridges for bromide was retained, that is 25 mM versus the expected 0.9 mM.

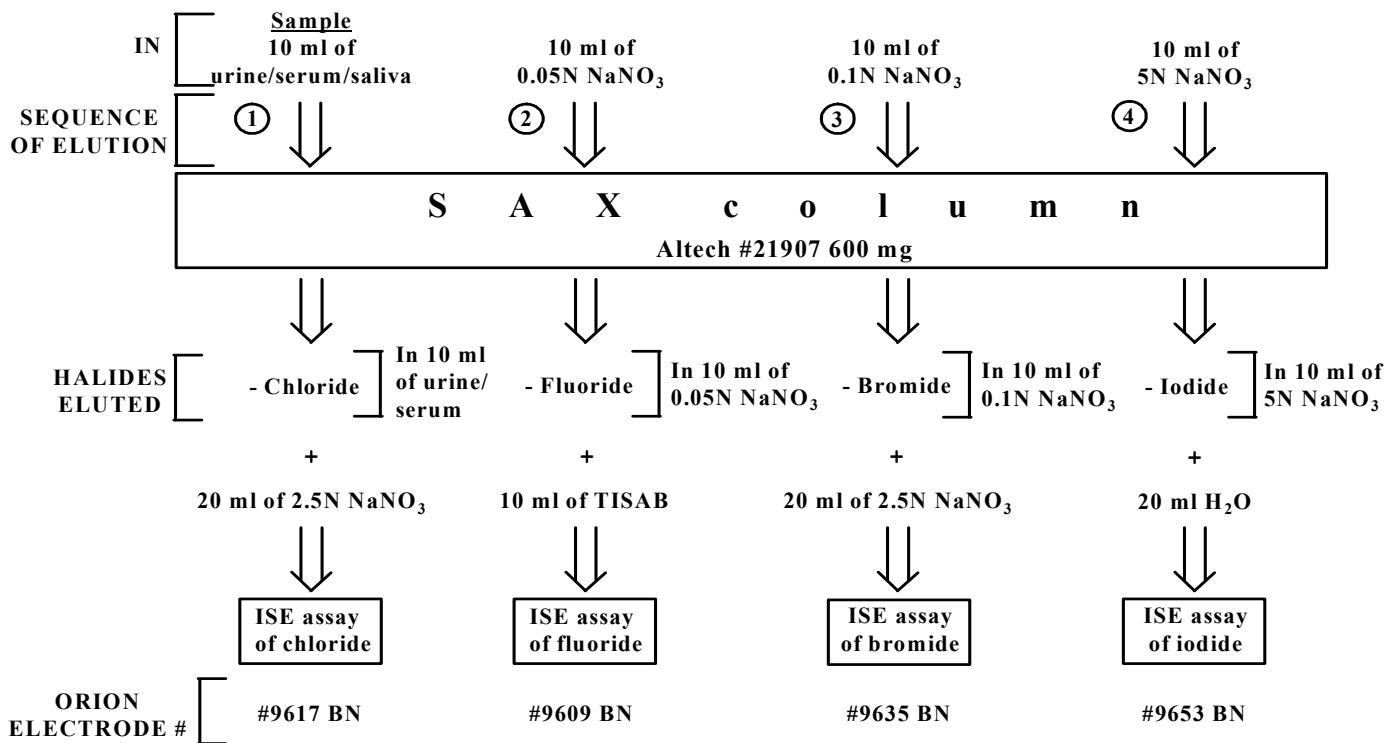
With a NaNO₃ concentration of 0.05 M in the urine sample, only 36% of the bromide was eluted with the urine fraction, with more than 60% remaining on the column; the second elution with 10 ml of 5N NaNO₃ contained only 76% of the iodide, with 24% remaining on the column. The retained iodide could be eluted with a second elution using 10 ml 5N NaNO₃. A minimum concentration of 0.2 M NaNO₃ in the urine sample was required in order to elute 100% of the iodide in the

second pass, but 20% of the bromide was present in the iodide fraction at that concentration. At 0.3 M concentration of NaNO₃, 90% of the bromide was eluted with the urine sample. At 0.4 M, we obtained quantitative recovery of bromide in eluted urine and quantitative recovery of iodide in the 5N NaNO₃ fraction. The ideal concentration of NaNO₃ in the urine samples was 0.4 M.

Above 0.4 M NaNO₃ in the urine samples, increasing amounts of iodide were recovered in the eluted urine with up to 30% of iodide in the eluted urine at the 1.0 M NaNO₃ level. We chose 0.4 M NaNO₃ in the urine samples for the simplified procedure. Under those conditions, bromide was completely eluted with the urine sample, with quantitative recovery of iodide in the second elution with 10 ml 5N NaNO₃. Since bromide possesses a much higher relative selectivity than fluoride and chloride for the SAX resin, complete elution of bromide was evidence of complete elution of chloride and fluoride in the eluted urine. The simplified ISE procedure is outlined in Figure 7.

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Figure 2
Flowchart Describing the combined Measurement of the Different Halides in the Same Sample



This chart describes the combined measurements of chloride, fluoride, bromide, and iodide in the same urine/serum/saliva sample by prior chromatography on the anion-exchange resin cartridge SAX 600 mg fitted with 10-milliliter plastic syringes in a positive displacement manifold. (From reference 8)

high capacity of the SAX 600 mg cartridges for bromide and iodide, we retested the capacity of the SAX cartridges for iodide and bromide with the 4-step procedure outlined in Figure 2, using increasing amounts of iodide and bromide. The information supplied by Altech Associates grossly underestimated the capacity of the SAX 600 mg cartridges. Up to 10 mM iodide (1,270 mg/L) were completely retained without breakthrough bleeding in the eluted urine, the 0.05 N and 0.1 N NaNO₃ fractions. All the iodide added was recovered in the 5 N NaNO₃ fraction. That is 10 times the maximum amount of iodide expected from theoretical consideration. The same high capacity of the SAX cartridges for bromide was observed even when 25 mM bromide (2,000 mg/L) was added to the urine samples, that is 25 times the expected capacity (Table 3). We have no explanation for this discrepancy between the data supplied by Altech and the data obtained at the Optimox Potentiometric R&D Laboratory.

We confirmed data supplied by Thermo Orion regarding the specificity of the iodide electrode from interference by chloride and bromide. Orion reported a chloride/iodide molar ratio of 1 million and a bromide/iodide ratio of 5,000 in order for the interference from chloride and bromide to occur in the ISE procedure of iodide. We measured a mean value of 582,000 for chloride and 5,530 for bromide (Table 4). The interference in the iodide assay due to chloride and bromide was non-linear as can be seen in Table 4. The relative interference increased along with the concentrations of chloride, whereas it decreased when bromide concentrations increased.

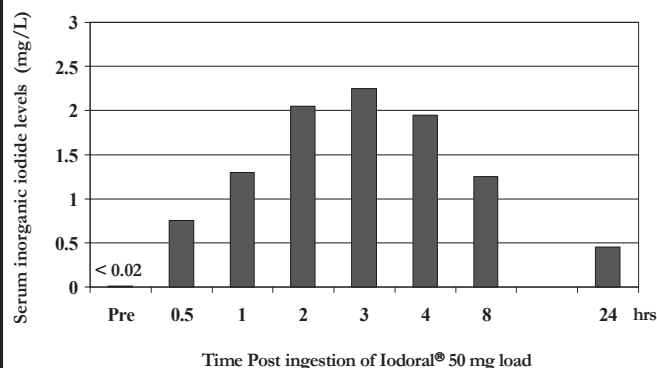
Reliability: The reliability of an assay depends on its sensitivity, specificity, accuracy, and precision.^{19,20}

Sensitivity: The theoretical limit of sensitivity achievable with ISE assay of urinary iodide is set by the sensitivity of the iodide selective electrode itself. From data supplied by Thermo Orion, the sensitivities of the ISE electrodes for halides are: 5×10^{-8} M for iodide; 10^{-6} M for fluoride; 5×10^{-6} M for bromide; and 5×10^{-5} M for chloride. The iodide selective electrode is by far the most sensitive, being 20 times more sensitive than the ISE electrode for fluoride, 100 times more sensitive than for bromide, and 1,000 times more sensitive than for chloride. Expressed as mg/L, the iodide selective electrode is sensitive down to 0.006 mg/L.

In achieving this theoretical sensitivity, other conditions are important. First, the sensitivity of the standard curve, which is defined as the smallest amount of iodide

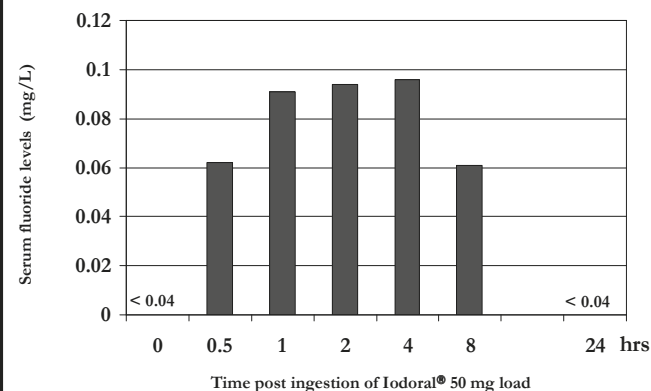
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Figure 3
Serum Profile of Inorganic Iodide Levels post Iodoral® 50 mg



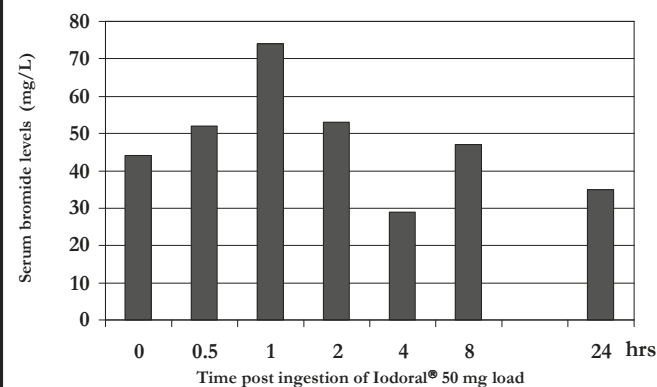
Mean value of six normal female subjects. (Modified from reference 8)

Figure 4
Serum Profile of Fluoride Levels post Iodoral®



Mean value of six normal female subjects

Figure 5
Serum Profile of Bromide Levels post Iodoral®



Mean value of six normal female subjects

Table 2

Chromatography of Halides Using the Altech SAX 600 mg Cartridge in a Positive Displacement Manifold PDM-20: Percent Recovery of Fluoride, Chloride, Bromide, and Iodide in Eluted Fractions of Increasing NaNO₃ Ionic Strength

Eluted Fractions (10 ml each)	Percent Recovery			
	Chloride	Fluoride	Bromide	Iodide
Eluate	≥98%	<1%	<1%	<1%
0.05 N NaNO ₃	<2%	≥98%	<1%	<1%
0.1 N NaNO ₃	<1%	<1%	≥98%	<1%
5.0 N NaNO ₃	<1%	<1%	<1%	≥98%

dence limit, is a limiting factor. In order to compute the standard curve, (the dose response curve), the EMF expressed in millivolts (mV) was plotted on the Y-axis against the logarithm of increasing amount of iodide, from 10⁻³ M to 10⁻⁸ M on the X-axis. The iodide selective electrode was extremely sensitive (Figure 8), with a linear response from 10⁻³ M to 10⁻⁷ M. A mean D EMF of 61 mV per decade was observed from 10⁻³ M to 10⁻⁷ M, but from 10⁻⁷ M to 10⁻⁸ M, the standard curve became non-linear with only 36.2 mV per decade.³

Specificity: We have previously validated the specificity of the ISE method by comparison of values obtained with ICP-M.S.³ In the simplified procedure, the addition of 0.4 M NaNO₃ to the urine sample resulted in the removal of the interfering halides, chloride and bromide, in the eluted urine prior to desorption of iodide with 10 ml 5 N NaNO₃.

Precision and Accuracy: The precision and accuracy of the 2-step method were tested by measuring 5-6 replicates of a urine collection from a subject not on

iodine supplementation, prior to and following addition of increasing amounts of potassium iodide to aliquots of the urine collection from 1 mg iodide to 50 mg iodide. The data are displayed in Table 5. Regarding accuracy of the 2-step procedure, percentage recovery of added iodide were 102% for 1 mg iodide; 92% for 10 mg iodide; 100% for 30 mg iodide; and 102% for 50 mg iodide. Regarding precision, the coefficient of variation of 5-6 replicate measurements were 7% for 1 mg iodide; 12% for 10 mg; 7.4% for 30 mg; and 6.3% for 50 mg iodide. The same experiments for accuracy and precision were performed with the 4-step method and displayed in Table 5 for comparison.

Methodology

The procedure described here is for the measurement of iodide in the 24-hour urine collection for the iodine/iodide loading test. The test is performed in order to assess whole body sufficiency for iodine prior to and following orthiodosupplementation.¹² However, it can be used also to measure iodide in spot urine samples.

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Table 3

Elution Profile of Iodide and Bromide

Eluted Fractions	Percent Bromide Recovered (mean of triplicates)	Percent Iodide Recovered (mean of triplicates)
Eluted urine	<0.1%	<0.1%
0.05 N NaNO ₃	<<0.1%	<0.1%
0.1 N NaNO ₃	99.2%	<0.1%
5.0 N NaNO ₃	<0.1%	98.6%

Elution profile of iodide 10 mM (1,270 mg/L) and bromide 25 mM (2,000 mg/L) on the SAX 600 mg cartridge using a step gradient elution with increasing concentration of NaNO₃ as outlined in Figure 2. (From reference 8)

Table 4
Interference of Increasing Concentrations of Chloride and Bromide Added to Water in the ISE Measurement of Iodide

Chloride Added	Iodide Measured*	Interference**
100 mM	0.15 μ M	670,000
500 mM	0.78 μ M	641,000
1,000 mM	2.3 μ M	435,000
Mean = 582,000		
Bromide Added		
10 mM	2.2 μ M	4,545
100 mM	18.6 μ M	5,380
1,000 mM	152 μ M	6,670
Mean = 5,530		

* Mean of triplicates

** Molar ratio of added halide/iodide measured

Iodine/Iodide Loading Test: If the patient is on orthiodosupplementation, he/she should stop ingesting iodine 24-48 hours prior to the loading test. The following instructions are presented as guidelines only:

- 1) Discard the first morning urine of Day 1.
- 2) Take four tablets of Iodoral[®] 12.5 mg with a glass of water.
- 3) Collect all urine samples for 24 hours following ingestion of the loading dose. Include the first morning urine on Day 2. A 3-liter plastic bottle appropriate for the 24-hour collection can be obtained from VWR Scientific Product (PN# 60872-564). The 16-ounce urine collection cup is available from General Bottle Supply Co., phone (323) 581-2001 (PN# T31216-1).
- 4) At the end of the 24-hour collection, shake the 3-liter bottle well. Measure volume of urine in the 3-liter bottle by looking at markings on side of bottle, and pour 1-2 ounces into a 2-ounce plastic bottle (VWR Scientific Product, PN# 16126-041). Two plastic 2-ounce bottles should be supplied in case the 24-hr urine volume is greater than 3 L.
- 5) If the 24-hour urine volume exceeds 3 L, then:
 - a) Measure volume of urine in the 3-liter bottle

by looking at the markings on the side of bottle. Pour 1 oz. of urine in the 2-ounce plastic bottle. Write the name, date, volume of urine on the 2-ounce bottle.

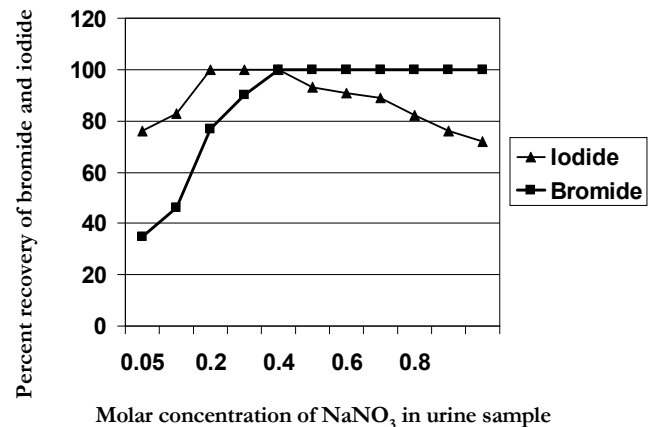
- b) Discard urine in the 3-liter bottle. Use the same 3-liter bottle to continue collection.
- c) At the end of 24-hour collection, measure urine volume again and repeat steps 5a and 5b.
- d) When the form is completed, under total volume, write: "Collected in 2 parts, both sample bottles enclosed."
- e) The concentration of iodide in the 24-hour urine will be the sum of both values obtained in the two specimens.
- f) If only one of the two sample bottles is needed, return the empty one with your package.

We use the food grade coloring FDC Green #3 at a concentration of 1 ml of a 1% solution per liter of urine.³ It is used for its antiseptic properties and also as a marker for spent cartridges. Having two benzene rings in its molecule, FDC Green #3 forms a very strong hydrophobic interaction with the benzene rings in the SDB backbone of the SAX. FDC Green #3 was retained on the column, resulting in a clear eluate. Spent

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Figure 6

Effect of Increasing Concentrations of NaNO₃



Effect of increasing concentrations of NaNO₃ in urine samples containing 300mM chloride, 12.5 mM bromide, 2 mM fluoride, and 0.1 mM of iodide on recovery of bromide in the eluted urine and the recovery of iodide in the second pass of 10 ml 5N NaNO₃ through the Altech cartridges (#21907) using the PDM shown in Figure 2.

could be identified by the greenish-blue color of FDC Green #3 retained on the resin of the cartridges.

Equipments and Materials: The equipment, accessories, and materials needed for the ISE procedure are displayed in Table 6. We recommend the Thermo Orion Meter #720-A Plus connected to the Thermo Orion iodide electrode 9653 BN, which is very sensitive for iodide (See Figure 8). The 9-position magnetic stirrer from Corning is available from Spectrum Chemical (Gardena, CA). The SAX cartridges are available from Altech Associates. The specifications are SAX cartridges #21907 Maxi Clean SPE 600 mg.

ISE Procedure: A urine sample from the 24-hour collection is transferred into a supplied 45 ml plastic vial with a snap top containing a tablet of NaNO₃ and FDC Green #3. The volume of urine must reach the level of the outer ring on the plastic vial (35 ml). After dissolving the tablet into the urine (0.4 N NaNO₃ final concentration), 10 ml of the urine containing 0.4 N NaNO₃ are aspirated into 10 ml plastic syringe (BD #301030). Do not discard the remaining 25 ml of urine in the plastic vial until you have satisfactorily completed the ISE measurement of iodide. The Altech SAX cartridge (#21907) is then fitted at the tip of the syringe. The syringe/cartridge combination is placed into the PDM-6 unit for elution. The speed of elution is set at 3 ml/min.

The eluted urine contains chloride, fluoride, and bromide, and it is discarded. The retained iodide on the SAX resin is then eluted with 10 ml 5 N NaNO₃ and collected into a 30 ml glass beaker containing 20 ml of purified water. The concentration of iodide is measured

under continuous stirring with the Orion ISE (#9653 BN) connected to the Orion Meter 720A-Plus which is programmed with the units of milligrams iodide per liter for the dose response curve.³ Figure 8 in this manuscript can be used as a guideline when setting up the dose response curve. Over the last five years, Optimox Potentiometric R&D Lab has used five different ISEs from Orion. The dose response curves for these electrodes are almost superimposable. These electrodes are very reproducible.

Physicians who do not want to invest in the equipment, time, or personnel required for the measurement of iodide *in situ* but wish to perform the initial step in the separation of iodide from the other halides can mail the SAX cartridges containing the iodide fraction to a laboratory familiar with this procedure. From preliminary testing at the Optimox Potentiometric R&D Lab, the retained iodine in the SAX cartridge is stable for up to 10 days at room temperature, as long as the hydrated resin does not dry out. To prevent drying of the cartridges, tight plugs are provided for both ends of the cartridge.

If the health care provider chooses the above option, the only equipment needed would be the PDM-6. There would be no need for glassware and pipetting devices. We are planning to offer kits with six cartridges, six syringes, and six plastic 45-milliliter vials with caps containing one tablet of NaNO₃ and FDC Green #3. The NaNO₃ concentration will be 0.4 N when the NaNO₃ tablet is dissolved in 35 ml of urine. The cartridges will have a code number so that the physician can supply the cartridges to another laboratory for

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Table 5

Accuracy and Precision of the ISE Assay of Iodide Using the 4-Step versus the 2-Step Chromatography on the SAX Cartridges

Iodide Added	4-Step Procedure				2-Step Procedure			
	(n)	Mean	SD	CV	(n)	Mean	SD	CV
0 mg	(6)	0.052	0.012	23%	(6)	0.045	0.010	22%
1 mg	(6)	0.96	0.084	8.9%	(5)	1.02	0.07	7%
10 mg	(5)	10.1	0.34	3.4%	(5)	9.2	1.1	12%
30 mg	(5)	32.6	1.1	3.4%	(5)	30.2	2.2	7.4%
50 mg	(5)	54.8	7.8	14%	(5)	50.8	3.16	6.3%

Values are expressed as mg iodide/L urine.
n = number of replicate measurements
CV = coefficient of variation

the amount of iodine/iodide ingested and the total volume of the 24-hour collection are needed if you want the laboratory to calculate the percent excretion of the oral load.

Urine samples with known amounts of iodide will also be available for testing the accuracy of the procedure in the physician's office. If the SAX cartridges containing the iodide fraction are mailed to another laboratory, one sample out of every six samples could be an aliquot of urine with known amounts of iodide. In this way, the reliability of the results from that laboratory could be evaluated.

Discussion

Until recently, the essential element iodine was totally neglected by the medical profession to the point where physicians did not request a single test for urine iodide concentration during their whole medical career. What they learned about inorganic, non-radioactive iodine

was mainly from epidemiological studies in areas of the world with extremely severe deficiency causing neurological defect and mental retardation. A recent review on the history of iodine in medicine²¹⁻²³ revealed that the neglect and fear of iodine in medical practice did not originate from published studies with documented cases of serious adverse effects to inorganic, non-radioactive iodine. Instead, it originated from iodophobic misinformation, surprisingly resulting in two successive waves of medical iodophobia which may have caused more misery and death in the US than both World Wars combined.²⁴ The first wave lasted 15 years (1910-1925) and the second wave is still alive and well after almost 60 years (1948-2006).

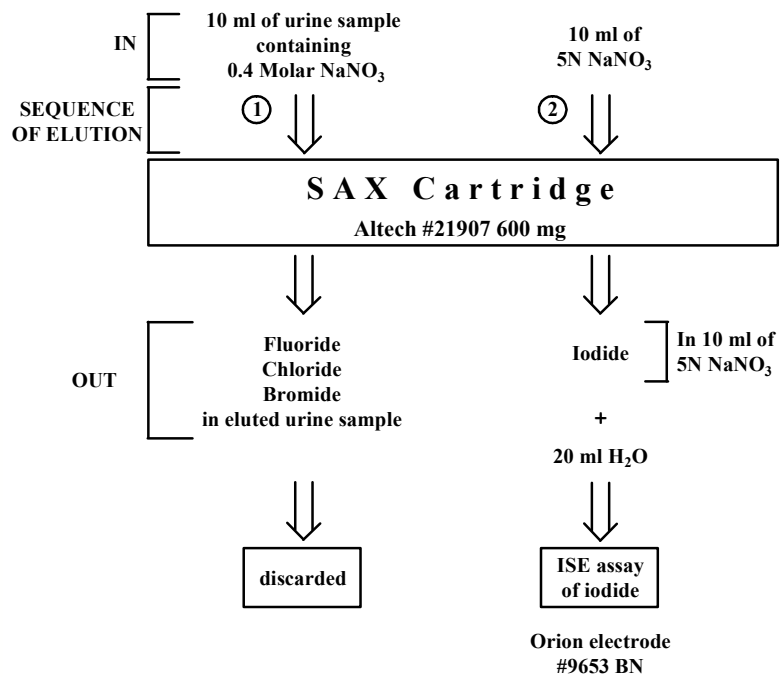
Over the past four years, in response to our publications emphasizing the safe and effective use of inorganic, non-radioactive iodine in medical practice,^{5,8-14,24,25} physicians and other health care practitioners have

implemented, safely and successfully, the orthoiodosupplementation program in their practice, including the iodine/iodide loading test prior to and following iodine supplementation. Currently, only two laboratories offer the measurement of urine iodide in the 24-hour urine collected during the loading test: FFP Laboratory (Hendersonville, NC) and Doctors Data (St. Charles, IL).

Both laboratories use the 4-step procedure with the PDM-20.⁸ The increased interest of physicians and other health care professionals in this program is exemplified by the fact that at the FFP Laboratory alone, more than 10,000 loading tests were performed over the last three and a half years.

We have simplified the ISE procedure in order to encourage more physicians and other health care professionals to implement the

Figure 7
The Simplified 2-Step Chromatographic Procedure



This procedure is used to separate the iodide fraction from the other halides prior to measurement by the ISE assay. NaNO₃ is added to urine samples to reach a concentration of 0.4 M.

orthoiodosupplementation program in their practices. Having various options for measurement of urine iodide by the ISE procedure with increasing degree of involvement by the health care practitioner, gives more flexibility in the assessment of whole body sufficiency for iodine during the follow-up of the patient on orthoiodosupplementation. We are planning to perform "field testing" of this simplified approach with the collaboration of health care professionals. The results of these tests will be the subject of a future article in *The Original Internist*.

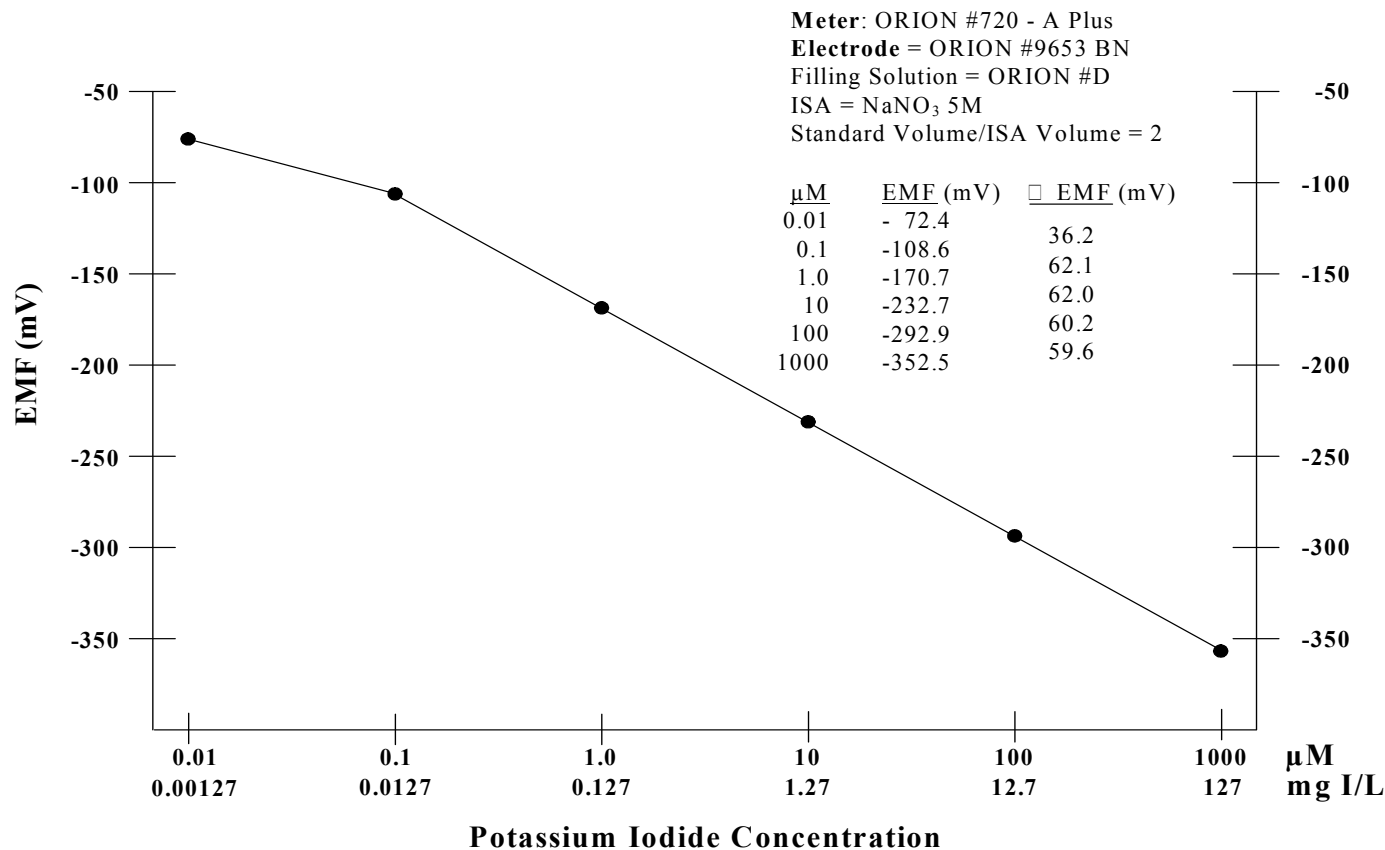
About the Authors

Guy E. Abraham, MD, is a former Professor of Obstetrics, Gynecology, and Endocrinology at the UCLA School of Medicine. Some 35 years ago, he pioneered

(Continued on next page)

Figure 8

Dose-Response Curve Observed with the Iodide-Selective Electrode



From reference 3

Belgium, 1976; the Senior Investigator Award of Pharmaciarnacia, Sweden, 1980.

Roxane C Handal, BS, is a graduate student at Boston University who spent a two week apprenticeship with Dr. Abraham at the Optimox Potentiometric R&D Lab.

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REFERENCES

- 1) Turner RG. "A micro colorimetric method for the quantitative estimation of iodine in blood." *J Biol Chem*, 1931; 88:497-511.
- 2) Taurog A and Chaikoff IL. "On the determination of plasma iodine." *J Biol Chem*, 1946; 163:313-322.
- 3) Abraham GE, Flechas JD, and Hakala JC. "Measurement of urinary iodide levels by ion-selective electrode: Improved sensitivity and specificity by chromatography on anion-exchange resin." *The Original Internist*, 2004; 11(4):19-32.
- 4) Cooper G and Croxson MS. "Chloride interference with use of an iodide-selective electrode for urinary I." *Clinical Chemistry*, 1983; 29:1320.
- 5) Abraham GE. "The historical background of the iodine project." *The Original Internist*, 2005; 12(2):57-66.

- 6) Yabu Y, Miyai K, Hayashizaki S, *et al*. "Measurement of iodide in urine using the iodide selective ion electrode." *Endocrinol*, 1986; 83:905-911.
- 7) Konno N, Yuri K, and Miura K. "Clinical evaluation of the iodide/creatinine ratio of casual urine samples as an index of daily iodide excretion in a population study." *Endocrine Journal*, 1993; 40:163-169.
- 8) Abraham GE. "Serum inorganic iodide levels following ingestion of a tablet form of Lugol solution: Evidence for an enterohepatic circulation of iodine." *The Original Internist*, 2004; 11(3):29-34.
- 9) Abraham GE. "Iodine supplementation markedly increases urinary excretion of fluoride and bromide." *Townsend Letter*, 2003; 238:108-109.
- 10) Abraham GE and Brownstein D. "Evidence that the administration of Vitamin C improves a defective cellular transport mechanism for iodine: A case report." *The Original Internist*, 2005; 12(3):125-130.
- 11) Abraham GE, Brownstein D, and Flechas JD. "The saliva/serum iodide ratio as an index of sodium/iodide symporter efficiency." *The Original Internist*, 2005; 12(4): 152-156.
- 12) Abraham GE. "The safe and effective implementation of orthoiodosupplementation in medical practice." *The Original Internist*, 2004; 11:17-36.
- 13) Flechas JD. "Orthoiodosupplementation in a primary care practice." *The Original Internist*, 2005; 12(2):89-96.

(Continued on next page)

- 14) Brownstein D. "Clinical experience with inorganic, non-radioactive iodine/iodide." *The Original Internist*, 2005; 12(3):105-108.
- 15) Thurman EM and Mills MS. *Solid-Phase Extraction. Principles and Practice*. Wiley-Interscience Publication, 1998; 123-160.
- 16) Tonacchera M, Pinchera A, Dimida A, *et al.* "Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter." *Thyroid*, 2004; 14:1012-1019.
- 17) Andrews J. "Thiocyanate and smoking in pregnancy." *J Obstet Gynaecol Br Commonw*, 1973; 80:810-814.
- 18) Chen Y, Pederson LL, and Lefcoe NM. "Exposure to environmental tobacco smoke (ETS) and serum thiocyanate level in infants." *Arch Environ Health*, 1990; 45:163-176.
- 19) Abraham GE. "Radioimmunoassay of steroids in biological materials." In: *Radioimmunoassay and Related Procedures in Medicine*. Proceedings of a symposium held by the International Atomic Energy Agency, Vienna, Austria, 1974; 3-30.
- 20) Abraham GE. "The application of natural steroid radioimmunoassay to gynecologic endocrinology." In: *GE Abraham's Radioassay Systems in Clinical Endocrinology*. Marcel Dekker, Inc., 1981; 475-530.
- 21) Abraham GE. "The history of iodine in medicine. Part I: From discovery to essentiality." *The Original Internist*, 2006; 13(1):29-36.
- 22) Abraham GE. "The history of iodine in medicine. Part II: The search for and the discovery of thyroid hormones." *The Original Internist*, 2006; 13(2):67-70.
- 23) Abraham GE. "The history of iodine in medicine. Part III: Thyroid fixation and medical iodophobia." *The Original Internist*, 2006; 13(2):71-78.
- 24) Abraham GE. "The Wolff-Chaikoff effect: Crying wolf?" *The Original Internist*, 2005; 12(3):112-118.
- 25) Abraham GE and Brownstein D. "Validation of the orthiodosupplementation program: A rebuttal of Dr. Gaby's

Table 6

Equipment, Accessories, and Chemicals Required for the Measurement of Urine Iodide by the ISE Procedure

Equipment	Manufacturer (PN)	Supplier (PN)	Approximate Cost	Supplier Phone #
ISE Meter	Thermo Orion (720A-Plus)	Cole Parmer (C-58810-50)	\$1,500	800-323-4540
Iodide Electrode	Thermo Orion (9653 BN)	Spectrum (246-25491)	\$650	800-772-8786
Magnetic Stirrer	Corning (440826)	Spectrum (338-21613)	\$1,300	800-772-8786
Positive Displacement Manifold PDM-6	Optimox (PDM-6)	Optimox (PDM-6)	\$900	800-223-1601
Accessories				
SAX Cartridges	Altech Associates (21907)	Altech Associates (21907)	\$150/50 cartridges	800-255-8324
Glassware Pipetting Devices	—	Spectrum	—	800-772-8786
Plastic Syringes (10 ml)	Beckton Dickinson (BD301030)	VWR BD301030)	\$165/850 syringes	800-932-5000
Chemicals				
Sodium Nitrate 5N	Spectrum (S-259)	Spectrum (S-259)	\$65/L	800-772-8786
Sodium Iodide 0.1 M	Thermo Orion (945306)	Cole Parmer (C057323)	\$90/L	800-323-4540
Electrode Filling Solution #D	Thermo Orion (900063)	Cole Parmer (C05713-36)	\$80/5 x 60 ml	800-323-4540