G. Urinary Iodine Measurement

An adequate dietary intake of iodine is required for normal thyroid gland hormone production and to maintain a euthyroid state. It follows therefore that the measurement of iodine intake from foodstuffs or medications has clinical relevance. In the clinical laboratory, iodine measurements are used primarily for epidemiological studies or for research *(3)*. To date, the major application of iodine analysis is to assess the dietary iodine intake of a given population *(3,397,398)*. This is an issue of considerable importance, since it has been estimated that iodine deficiency disorders (IDD) potentially affect 2.2 billion people throughout the world. Even in developed countries such as the USA and Australia, a decline in dietary iodine intake has been demonstrated, while borderline dietary intake has long been characteristic in much of Europe *(398,399)*.

As the majority of ingested iodine is excreted in the urine, the measurement of urinary iodine excretion (UI) provides an accurate approximation of dietary iodine intake *(399)*. In most circumstances the determination of UI provides little useful information on the long-term iodine status of an individual, since the results obtained merely reflect recent dietary iodine intake. However, measuring UI in a representative cohort of individuals from a specific population provides a useful index of the iodine level endemic to that region *(399,400)*. Besides estimating the UI concentration in people, other applications of iodine measurements include determining iodine in milk, food products and drinking water *(401,402)*. Iodine measurement in thyroid or breast tissue has been performed as part of research studies *(403)*. Since low inorganic iodine concentrations in serum (~ 1pg/dL) are associated with relatively abundant hormonal iodine, the measurement of plasma inorganic iodine (PII) has been restricted to research studies in pregnancy *(404)*.

1. Urinary Iodine (UI) excretion

The UI-excretion level from a population study can provide a relatively accurate estimate of the dietary iodine status of that population *(399,400)*. Iodine intake is best determined from a 24-hour urine sample, but logistics make it impractical to use such measurements for epidemiological studies. Differences in the dilution of spot urine specimens can be compensated for by expressing results normalized to urine creatinine as µg of iodine excreted/gram of creatinine *(405)*. The diurnal and seasonal cycles of iodine and creatinine urinary excretion are different. Therefore the ratio of iodine/creatinine can vary during the day or the time of year. In addition, there is no ideal substitute for the accuracy of a 24-hour urine collection, which can be difficult to obtain. However, the UI estimation of iodine intake is most important in developing countries where the iodine/creatinine index may be less satisfactory and where there is a lower creatinine excretion rate secondary to varying degrees of malnourishment *(406)*. It has also been shown that urinary iodine excretion can be variable even in healthy, well-nourished subjects. For these reasons, and to avoid errors introduced in the performance of different creatinine assays, the World Health Organization (WHO) has recommended that for epidemiological studies, the excretion of UI may be expressed as µg of iodine per volume (pg/dL or µg/l) of urine. Differences in urine dilution states inherent in obtaining a spot urine sample can be partially compensated for by using a large number (~50) of subjects in each study population. Recent reports suggest that the use of age and sex adjusted (UI/Cr) ratios in a fasting morning specimen comes close to the true (24 hour) iodine excretion if nutrition is generally adequate *(400,407)*. Although seasonal variations may not be as important in warmer climates, they do affect the results in Northern Europe where dairy milk is a major source of dietary iodine. In such populations, the practice of indoor feeding of cattle with mineral rich supplements results in higher UI excretion during the winter months. More recently it has been suggested that UI has a diurnal variation, with values reaching a median in early morning or 8-12 hours after the last meal suggesting that samples should be collected at these times *(408)*.

2. Dietary Iodine

In many countries, adequate dietary iodine intake is achieved through the iodization of salt but the availability of iodized salt is only mandatory in some developed countries and voluntary in many others. There is also evidence of a decline in iodine consumption in some industrial countries *(399)*. Diminished iodine intake can occur with vegetarian diets, particularly in areas where the fruits and vegetables are grown in iodine deficient soil *(409)*.

3. Units of UI Measurement

For epidemiological studies, iodine excretion is normally expressed as µg of iodine excreted. Conversion to equivalent SI units:

- µg/dL = 0.07874 µM/L
- 1.0 pM/L = 12.7 pg/dL.

4. Applications of Iodine Measurement

(a) Epidimiologic Surveys

The major application of iodine measurements is for epidemiological surveys. The recommended daily iodine intake is: - 90 µg/day for children, 150 µg/day for adults and 200 µg/day for pregnant or lactating mothers. The suggested norms for UI excretion as an index of the severity of iodine deficiency are shown in Table 10 *(398)*.

Table 10. Urinary Iodine Excretion and Iodine Deficiency

*Iodine Deficiency	None	Mild	Moderate	Severe
UI µg/L	>100	50-99	20-49	<20
Goiter prevalence	<5%	5.0-19.9%	2~29.9%	>30%

*IDD Newsletter Aug 1999 15: 33-48

(b) Pregnancy and the Neonate

Fortunately, the occurrence of severe iodine deficiency that leads to endemic cretinism has been reduced as a result of dietary iodine supplementation programs. However, iodine deficiency still persists in large areas of the globe. The situation where dietary iodine deficiency may have more serious consequences is in the pregnant woman, where maternal iodine deficiency can compromise the thyroid status of the fetus and newborn child *(2,410)*. Reports on the variation in UI excretion during pregnancy differ. Some studies have reported a decline or no change, while others have shown an increase *(47,411-413)*. These differences may reflect variations in the dietary iodine supply *(414)*. However, the use of urinary iodine to estimate iodine sufficiency during pregnancy can be misleading, since pregnancy causes an increase in the iodine excretion rate. This results in a relative increase in the urinary iodine concentration, thus giving a false sense of adequacy of iodine nutrition *(415)*. It has been shown when dietary iodine intake is inadequate during pregnancy, that there is evidence of thyroidal stress, an increase in both thyroid volume and serum Tg and a relative decrease in FT4 *(47)*. Administration of iodine to pregnant mothers results in increased UI excretion and a reversal of the observed iodine deficient thyroidal changes. The importance in avoiding any compromise in thyroid function during pregnancy was recently emphasized by the report that children of even mildly hypothyroid mothers can develop defects in neuropsychological development *(64,65)*. This finding is consistent with earlier reports that plasma inorganic iodine (PII) declines during pregnancy. Early methods of measuring (PII) were based on the administration of a tracer dose of ^{131}-I to patients and measuring the specific activity of the radioisotope in serum and urine *(405)*. Other methods depended on the ratio of iodine to creatinine in serum and urine *(405,416)*. A recent study using perchlorate digestion and the formula PII = Total Serum Iodine - Protein Bound Iodine concluded that, at least in iodine sufficient areas, there was no trend for PII values to be depressed during pregnancy *(404)*.

(c) Excessive Iodine Intake

It is well known that excessive iodine intake may, in susceptible individuals, lead to the inhibition of thyroid hormone synthesis (the Wolff Chaikhoff effect) and can be of iatrogenic origin *(417,418)*. A similar excess of iodine intake by previously iodine-deficient individuals with thyroid autonomy may result in hyperthyroidism (the Jod Basedow effect) *(398,420)*. Population based dietary iodine intake programs can influence the form of

thyroid disease that occurs. This is particularly true for hyperthyroidism, with toxic nodular goiter being more prominent when iodine intake is low, and Graves' disease more prominent when iodine intake is high. However, it has been shown that a program of controlled dietary iodine intake can, after a transient increase in hyperthyroidism in the first year, cause a decrease in both toxic nodular goiter and Graves Disease if followed over a period of time *(421)*. Differences in disease presentation can also alter the epidemiological profile of thyroid cancer with a relative increase in papillary thyroid carcinoma together with an improved prognosis when the iodine supply is increased *(422)*.

Fear of the side effects of excess iodine has impeded the introduction of programs of iodine prophylaxis or even the possibility of administering iodine following accidental release of radioactive iodine. There is however, general agreement that the benefits of iodine administration far exceed the risks from excessive iodine exposure *(398)*. Thus the requirement for iodine measurements in the assessment of iodine excess states may exceed that for iodine deficiency. Iodine excess can result from the use of iodine rich medications such as the commonly prescribed cardiac antiarrythymic drug amiodarone or antiseptics containing iodine (Guideline 5) *(75,418,419,421,423)*. The thyroidal consequences of amiodarone therapy may depend on the underlying dietary iodine status of the area where the patient resides. Hypothyroidism is more frequent where dietary iodine intake is high, such as in the USA, and hyperthyroidism is more frequent where the intake is low, such as in parts of Europe *(424)*.

Excess dietary iodine intake has also been implicated in the increased prevalence of autoimmune thyroiditis or increase in thyroglobulin antibody positivity following iodine prophylaxis. This may be due to increased antigenicity of more highly iodinated forms of thyroglobulin *(425,426)*. The assessment of iodine excess is usually made with a 24hr urine collection. It should be understood that organic iodine present in radiological contrast material can be taken up into body fat. The slow release of iodine from body fat stores has been associated with a high UI excretion rate that can persist for several months following the administration of this contrast material. *(427)*.

5. Iodine Methodology

Methods that measure iodine content in biological specimenshave traditionally relied on the conversion of organic iodinated compounds to inorganic iodine and the removal of potential interfering substances (eg. thiocyanate) that can interfere with the colorimetric measurement of the inorganic iodine *(428)*. The procedure involves a preliminary digestion step followed by the colorimetric estimation of iodine through its catalytic action in the Sandell-Kolthoff (SK) reaction. In this reaction, Ce^{4+} (ceric ions) are reduced to Ce^{3+} (cerous ions) in the presence of As^{3+} (arsenious ions) that are then oxidized to As^{5+} (arsenic ions) producing a change in color from yellow to colorless. Following a short incubation period, this color change can be determined colorimetrically. As this reaction is time dependent, some reports suggest stopping the reaction with the addition of ferrous ammonium sulfate and performing the colorimetric readings at a later time. Further modifications of the SK reaction can produce a kinetic assay by altering the ratio of Ce/As ions. This kinetic method approach can increase the sensitivity of the assay *(429)*. The problems associated with the removal of interfering substances such as thiocyanate in the SK reaction have been previously mentioned, and a report comparing 6 methods for iodine analysis attributed much of these interferences with the SK reaction to inadequate digestion procedures *(428)*. Two major methods of sample digestion, dry ashing and wet ashing are routinely employed.

(a) Dry Ashing

The dry-ashing technique was first introduced in 1944 and subsequently modified. The method involves the preliminary drying of specimens in an oven at 100°C. The dried residue is then incinerated in the presence of strong alkali (KOH/K_2CO_3) for approximately 3 hours at 600°C. The ash is then reconstituted in distilled H_20 and the iodine content measured colorimetrically as described above. This is a somewhat time consuming and expensive method requiring thick-walled pyrex test tubes to withstand the high temperatures and a muffle furnace, ideally equipped with microchip temperature control. However, it does yield excellent results not only in urine samples but is also suitable for measuring the iodine content of foodstuffs and tissue samples that

require complete digestion. Strict temperature control is particularly useful in preventing iodine loss should the temperature drift above 600°C or if the time of incineration is extended *(429,430)*. It is also important that the iodine standards be subjected to incineration, as the added KOH is known to reduce the sensitivity of the SK reaction based assay. These methods were developed for the determination of protein bound iodine (PBI) used to measure thyroid hormones before the advent of specific radioimmunoassays for T4 and T3. As samples are incinerated together in a muffle furnace, the dry-ashing procedure is particularly susceptible to cross-contamination by a high iodine-containing specimen. To overcome this possibility some investigators have suggested prior screening of samples to detect such specimens. The problem of cross-contamination is particularly problematical with the dry-ashing procedure but has the potential to affect all iodine quantitation methods. It is therefore desirable that the iodine measurement area be isolated and kept as far away from other laboratory activities, particularly those that might involve use of iodine-containing reagents. The aesthetics of handling and volatilizing large volumes of urine for epidemiological studies also makes the isolated laboratory desirable.

(b) Wet Ashing

The most widely used method of digestion is the wet-ashing technique first proposed in 1951, although this approach is controversial. In this method the urine specimens are digested using perchloric acid. This method has been automated. While the autoanalyser method has found widespread use, it does depend on the use of acid digestion and a dialysis module. The latter has been shown to be prone to significant interference by substances such as thiocyanate *(428)*. Several variations of the wet ashing method for iodine measurements have been developed. These are primarily aimed at simplifying the methods, reducing the labor cost and rendering the method more suitable for on site epidemiological studies. Various methods have been described which yield similar results to established methods *(431)*. In one such method, the authors indicate that a single technician can perform 150 tests per day at a cost of less than $0.50 each *(431)*. More recently, even simpler methods using either acid digestion or UV irradiation of samples have been described *(432)*. The wet-ashing technique has drawbacks in that perchloric acid and potassium chlorate are potentially explosive and their use requires the use of a dedicated and expensive fume hood. For this reason a less hazardous method of digesting urine samples using ammonium persulfate as the oxidizing agent was proposed. However, the use of ammonium persulfate was shown not to be a very efficient means for mineralizing iodinated compounds such as T3, T4, amiodarone etc. A further modification involving the incorporation of the digestion and reaction process into a microplate technology has been reported *(433)*. More recently an assay was developed in kit form that allows for a more rapid quantitative measurement of UI after charcoal purification. (Urojod, Merck KGaA, Darmstadt, Germany). This method appears simple to perform and has the potential to be used in the field for epidemiological studies or for occasional use in the assessment of excess iodine ingestion *(434)*.

(c) Sensitivity and Specificity of Iodine Methods

Assays using the SK reaction yield sensitivities between 10 and 40 mg/L that is more than adequate for UI measurement. Greater sensitivity has been reported using the kinetic assay (0.01pg/L) *(429)*. Reported sensitivities using inductively coupled plasma mass spectrometry (ICP-MS) technique is in the area of 2µg/L *(413,434)*. Providing the initial digestion is complete, the SK assay is very specific for iodine. However incomplete digestion can lead to interference by substances such as iodine-containing medications, thiocyanates, ascorbic acid or heavy metals such as Hg or Ag *(429)*. In expert hands the SK reaction yields excellent intra- and inter-assay precision with CV's < 5% routinely achieved. This is provided the digestion is adequately controlled so that the recovery of the iodine standard is 90 to 100% *(429,430,432)*.

(d) Non Incineration Assays

In addition to methods based on alkaline and acid digestion, other published methods for iodine determination include the use of bromine in acid conditions as a digesting agent or the use of ultraviolet radiation *(430,435)*. Iodine selective electrodes and mass spectrometry have been used to measure iodine in various fluids including urine *(436,437)*. In this case the iodine activity that is measured approximates the iodine concentration. A drawback to this method is that the electrodes become coated and require frequent polishing and other ions such as sulphite interfere. This approach is therefore not ideally suited for measurements in urine but can be used to

measure iodine in other fluids and extracts of foodstuffs. Although not suitable for routine UI measurement, the technique can be applied to the assessment of iodine overload in urine in patients treated with amiodarone or other iodine rich compounds *(437)*. As the electrode only responds to iodine and not to iodinated compounds, it can be a useful means of specifically measuring iodine in the presence of other iodinated compounds. Many other techniques that are clearly unsuitable for routine clinical use include nuclear activation analysis, or HPLC. One method that has been widely reported is the use of (ICP-MS) *(432,438)*. This method has been shown to have good agreement with conventional digestion techniques using SK quantitation *(432,433)*. However, the required equipment is expensive and not readily available. Isotope dilution analysis has been applied to the analysis of both urine and drinking water *(402)*. In vivo measurement of intrathyroidal iodine content has been achieved using X-ray fluorescencethat can have relevance to the assessment of patients with amiodarone induced hyperthyroidism *(419)*.

Guideline 55. Urinary Iodine Measurement

- The Technicon Autoanalyser is generally no longer commercially available, with the result that laboratories seeking to commence iodide measurement will need to develop manual in-house methods.
- Mass spectrometry is a simple and reproducible method which can be recommended if such equipment is already on site.
- Many simplified digestion methods incorporating SK colorimetry have been described.
- Wet-ashing reagents perchloric acid and potassium chlorate are potentially explosive and their use requires availability of an expensive fume hood. A less hazardous system using ammonium persulfate may be preferable
- Measurement of iodide in samples other than urine (eg tissues, foodstuffs) may still require the more conventional dry or wet-ashing techniques.
- Inter and intra assay CV should be < 10% and recovery of added iodide should be between 90 and 100%.
- In industrialized countries, clinical laboratories are most frequently requested to perform urinary iodide measurements to investigate iodide overload. One of the simplified methods outlined above, or a semi-quantitative kit is the method of choice.
- To facilitate uniformity in concentration units used to report urinary iodide excretion, UI should be expressed as µg Iodide /L of urine (µg/L).

6. Summary

Measurement of iodine in tissues and biological fluids is unlikely to play a key role in routine clinical biochemistry laboratories in the immediate future. However in view of the large number of individuals with IDD worldwide (2.2 billion affected) and recent reports that dietary iodine intake is declining in the United States and Australia, the assessment of UI as part of epidemiological studies will continue to be of considerable interest and importance. Reference laboratories will no doubt continue to use dry- or wet-ashing techniques, depending on availability of equipment and space. Recent recommendations that laboratories " have several different methods available to allow the user to select the one best suited to specific needs" would seem a prudent course for centers specializing in iodine measurements.