Scimetr. 2013 Oct;1(1): e14092. DOI: 10.5812/scimetr.14092

Published Online 2013 September 9. **Review Article**

A Review on Iodine Determination Methods in Salt and Biological Samples

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Received: August 07, 2013; **Revised:** August 20, 2013; **Accepted:** August 21, 2013

Context: Iodine is an essential micronutrient, particularly because of its role in the structure of the thyroid hormone. Many people live in iodine deficient regions of the world, and hence need dietary iodine supplement. Though, iodine analysis of biological samples, especially urine, is a method for the evaluation of iodine status in consumers. This paper presents a review of the most common methods used to determine iodine levels in salt and biological samples.

Evidence Acquisitions: We conducted a literature review of published English articles in various parts of the world using databases from PubMed, World Health Organization between 1934 and 2012.

Results: A total of 2030 articles were identified and after eligibility criteria based evaluation, 63 articles were included in this literature review. The titration method is the most commonly used method for quantitative evaluation. The two main methods used to determine iodine levels in urine and milk are as follows: The instrumental method and the colorimetric methods in association with Sandell and Kolthoff reaction.

Conclusions: Despite the high risk of bias of many included studies, the results suggested the Sandell and Kolthoff reaction methods to determine iodine in milk and urine, methods are now technically very simple and have been used by many laboratories to measure iodine levels for many years. The titration method remains the reference method to determine iodine concentrations in salt. Other methods should be standardized against the titration method. Dried whole blood spots Triglycerides (TG) and thyroid stimulating hormone (TSH) are sensitive indicators of iodine deficiency.

Keywords: Iodine; Urine; Milk; Serum; Sodium Chloride

1. Context

Iodine is an essential micronutrient for animals and humans. It is also necessary for the synthesis of thyroid hormones, including thyroxin (T4) and triiodothyronine (T3), and its deficiency causes poor mental and physical development in children and also goiter in adults (1, 2). Wide ranges of physiological activities in almost all organisms need thyroid hormones, which are critical for metabolism, initial growth and organ development, specifically in brain. Therefore, iodine deficiency during the critical periods of life can disturb synthesis of thyroid hormones and result in a metabolic deceleration and cause permanent brain damage (3). Iodine deficiency is a globally recognized problem and studies show that 30% of the world's population lives in areas with iodine deficient soil. Mild iodine deficiency in women may cause attention deficit hyperactivity disorder (ADHD) in their children (4, 5). Even a mild iodine deficiency may seriously affect a child's intelligence and function (6, 7). Congenital hypothyroidism is a serious condition that has a permanent effect on neurological function (8). Breast-fed infants are completely dependent on milk iodine concentrations in their mothers, which make them particularly vulnerable if their mothers are iodine deficient. A high rate of congenital hypothyroidism is usually associated with a recent decrease in iodine intake (9).

The world health organization (WHO) recommends a daily intake of 90μg of iodine for preschool children (up to 59 months), 120μg for schoolchildren (6 to 12 years), 150μg for adolescents and adults, and 250μg for pregnant and lactating women (10). However, consumption of excess iodine can cause health problems such as hyperthyroidism and thyroid autoimmune diseases (11). Investigations done on iodine concentrations in foods and drinking water, show that high amounts of iodine are found in seafood; however, this source of iodine intake is not usually sufficient to supply daily requirements especially in pregnant women (12, 13). People's daily requirement for iodine can be provided by iodized salt, which can provide iodine supplementation for sensitive groups such as pregnant women and young children. Insufficient daily iodine intake

Implication for health policy/practice/research/medical education:
In this article, the most common methods for iodine determination in salt and biological samples, including urine, serum and milk have been reviewed.

can arise from changes in salt fortification, storage, transport and cooking procedures (4, 13, 14). Thus tests to assess levels of iodine should ideally be determined in a variety of sources such as food (15), salt (16, 17), in biological samples (18), notably milk (19), serum (20) and urine (17, 21).

To date, many analytical methods are available for iodine detection and monitoring in salt and human samples (Table 1). However, some of the methods have the specificity and sensitivity to measure iodine in biological matrices and these methods are approved by WHO. This review presents an evaluation of the most common methods used to determine iodine in salt and biological samples, including urine, serum and milk.

2. Evidence Acquisitions

The methodology applied for this review includes searches of literatures through the MEDLINE/PubMed, WHO, Internet and compilation of all published studies between 1934 and 2012. We included any cross-sectional, longitudinal or review article in English that reported national or international iodine determination in salt, urine, milk and serum. More than 2030 studies from various parts of the world were reviewed. 1955 articles were excluded after the title and abstract assessment. Full text of retrieved articles was evaluated by two reviewers, and 12 articles that were irrelevant or duplicate were excluded. Finally, 63 articles were included in this literature review. The keywords used to search were iodine determination, salt iodine, urinary iodine, milk iodine, iodine deficiency, serum iodine.

3. Results

3.1. Iodine determination in salt

Ideally, internal and external quality control and assurance programs in and out of the production site respectively should monitor iodine determination in salt. The titration method, or similar methods should be used to determine iodine content in salt quantitatively (10, 25). Quality assurance is a wide-ranging concept that includes all

Table 1. Summary of Iodine Determination Methods in Salt and Biological Samples

Samples	Methods	References
Salt	Titration	(5, 22, 23)
	Rapid testing kits	(22, 24)
	Instrumental neutron activation analysis (INAA)	(4)
	Electrochemical detection (ED)	(4)
	Flow Injection analysis (FIA)	(4)
Urine	Sandell–Kolthoff reaction: Autoanalyzer, Flow Injection, microplate,	(4, 25-27)
	Inductively coupled plasma mass spectrometry (ICP-MS)	(4,28)
	UV–vis spectrometry	(4)
	Flow injection analysis (FIA)	(4)
	Intracavity laser absorbance	(4)
	Rapid test kit	(5)
	Kinetic colorimetry	(2,4)
	Ion selective electrodes (ISE)	(4)
	Electrochemical Detection (ED)	(4)
	Instrumental neutron activation analysis (INAA)	(4)
Milk	Sandell–Kolthoff reaction	(4, 26, 27)
	Inductively coupled plasma mass spectrometry (ICP-MS)	(4, 29)
	Inductively coupled plasma optical emission spectrometry (ICP-OES)	(4)
	Instrumental neutron activation analysis (INAA)	(4)
	Atomic absorption spectrometry (AAS)	(4)
	Ion selective electrodes (ISE)	(4)
	Electrochemical Detection (ED)	(4)
Serum	S-K reaction	(4)
	Inductively coupled plasma optical emission spectrometry (ICP-OES)	(4)
	Ion selective electrodes (ISE)	(4)

factors, which either individually or collectively affect the quality of a product. It is used to control the quality of equipment, product design, supplies and logistics, management and human resource development, and includes all elements of design (30). Salt fortification is usually done with potassium iodide (KI) and potassium iodate (KIO3), because of the good iodine availability and its low cost (25, 30). However, potassium iodate is recommended mainly for salt by international organizations such as the WHO and the united nations children's fund (UNICEF), and to be stored under warm and humid conditions. Iodate is recommended because it is much more stable than iodide (KI) (30, 31). According to the WHO, UNICEF, and the international council for the control of iodine deficiency disorders (ICCIDD) (10) 20% of the iodine in salt is lost from production to a household, and another 20% is lost during cooking before consumption. The average salt intake is 10g per person per day. Hence to provide 150μg daily requirement of iodine for each person, the salt iodine concentration at the point of production should be (20–40 mg of iodine per kg of salt) (31). Today, salt iodization is the most commonly used method to control and eliminate iodine deficiency disorders (IDDs) (13).

Quantitative and qualitative evaluations of iodine in salt can be determined by titration and rapid test kits, respectively (22). Effective quality control in the production of iodate salt requires a simple, reliable and cost-effective analysis method. Several analytical techniques have been applied to determine iodate in table salt. For example, inductively coupled plasma mass spectrometry (ICPMS) (32), radiochemical neutron activation analysis (33) and capillary electrophoresis (34) have all been used to determine trace amounts of iodide. ICPMS and radiochemical neutron activation analysis techniques meet the requirements for sensitivity and accuracy for the determination of iodide but these techniques are not easily applicable because they need a high level of specialization and are expensive (28). Ion chromatography technique is used for direct determination of iodide (35). High levels of chloride in the medium affect the efficiency of iodine chromatography but this is can be avoided by adding chloride to the eluent. However, the technique is inappropriate for routine use because of insufficient sensitivity to detect iodine, as observed in conductivity, amperometry or potentiometry (36).

Two processes have been developed to overcome these detection problems, namely post-column reaction and spectrophotometry to convert iodide to a more detectable organic derivative (37). The problem with these methods is that they do not have sufficient sensitivity or detection capacity, and hence, are not able to detect trace levels of iodine (28). Most laboratories use the iodometric titration method to measure levels of iodine, but some use the potentiometric method (WYD model, China) (17).

3.1.1. Titration method

Titration is the most frequently used method to determine quantities of iodine in salt because of its accuracy, relatively easy to use and incurs low cost. This method is recommended at various levels of a distribution system. Internal and external quality control measures are necessary once a method has been established (10). The titration method for potassium iodate was introduced in 1979 (38). Depending on the form of iodine (iodate or iodide), different salt iodine testing methods are needed in the fortification process. If a salt sample is fortified with potassium iodide, the iodometric method will not detect iodine contents, and vice versa. In cases of an unknown form of iodine in salt, a simple spot check method can be used for verification (25). In this method first, the iodine content of salt can be determined by liberating iodine from a salt sample then by the titration of iodine with sodium thiosulphate using starch as an external indicator (10, 39). However, the titration method is not recommended for routine national monitoring purposes because it's a time-consuming process (10).

3.1.2. Rapid salt testing kits

Rapid test kits are applicable to both qualitative and semi quantitative estimations of iodine content (25). These kits are rapid, simple and easily applied in a field setting, and need no training of a chemistry laboratory personnel. Spot tests can be used at sites of production, distribution, retail, and household. They are especially suitable for small-scale salt producers who may not be able to achieve the level of sophistication needed to establish more quantitative laboratory titration methods (40). There are various spot tests available, all of which use the same common reaction mechanism, a starch based reagent solution that produces a blue color when iodine is present in a salt sample (25). Overestimates of iodine content and false-positive results may occur from the use of contaminated instruments such as spoons and test plates (40). However, some major salt companies use potassium iodide instead of iodate, while UNICEF salt kits are only able to detect iodate so these tests need to be checked for false-negative results and the shelf life of the kits needs to be monitored (25). Nevertheless, the titration method must support results of rapid test kits because they cannot provide consistent estimates of iodine content (10).

3.2. Iodine determination in urine

The implementation of IDD control programs uses the principal indicator of urinary iodine rather than thyroid size, TSH and thyroglobulin. However, thyroid size is more useful as a baseline assessment of the severity of IDD and it has a role in assessing the long-term impact of control programs (10). As adequate iodine is essential for neurocognitive development during fetal

development and childhood, target groups for studying iodine levels are pregnant women (4) and newborns (23). Over 90% of dietary iodine eventually appears in the urine, and samples are easy to collect and thus available for analysis (21 , 41). As a result, urinary iodine excretion can be a good indicator of current dietary iodine intake for IDD. Urinary iodine excretion can vary in individuals from day-to-day and even within a day. However, more accurate estimates of iodine levels among populations can be determined by a median evaluation of urinary iodine excretion from at least 30 individuals. The median value, rather than the mean, for a sampled population is often considered as an indicator, as urinary iodine concentrations from populations are usually not normally distributed. Iodine deficiency in groups is diagnosed according to specific cut-off values of median urinary iodine concentrations (Table 2). Moreover, not more than 20% of samples should be below 50μg /L (3 , 10).

A suitable method for urinary iodine measurement should consider high accuracy, reliability, high speed, technical demand, low complexity of instrumentation, independence from sole source suppliers, availability of high-quality reagents, safety and low cost (10). All laboratories should have a clearly defined quality control procedure. Commercial standards should provide the basis for accurate measurement, recovery tests and to set a minimum detection limit (10, 17, 24). Some studies showed that iodine spiked samples had recovery between 94% to 103% (42, 43). Inter-batch variation could be established by using duplicate analyses of control samples within each time period of some days. Intra-batch variation could be established, as a measure of reliability (precision), by at least 10% analyses at low, medium and high iodine concentrations. Participation in the ensuring the quality of urinary iodine procedures (EQUIP) program to have external quality assurance program is strongly recommended for laboratories testing urinary iodine. To help and assess the accuracy of UI levels in laboratories around the world, the Centers for Disease Control and Prevention (CDC) sends three samples three times per year to participating laboratories for analysis (10, 24, 32).

Random morning spot UI concentration is the most commonly used method for evaluating UI assessment. Twenty-four hour urine samples are unsuitable, as they are difficult to obtain where accurate sample collection is critical. Creatinine has been used as a marker for sufficiency assessment in a 24-h urine collection. Urinary iodine to Creatinine ratio is difficult and unworkable in epidemiological studies. Indeed, since amounts of urinary creatinine excretion are dependent on protein intake, nutrition quality and age, urinary creatinine concentrations can vary in individuals (10, 44). Many studies have shown that iodide and iodine may be quantified by a variety of methods, including gas chromatography-mass spectrometry, ICP atomic emission spectrometry, UV-Vis spectrophotometry, catalytic spectrophotometric methods, atomic absorption spectrometry, ICP-MS, capillary electrophoresis and chromatography (15, 26, 45).

Table 2. Epidemiological criteria for assessing iodine nutrition in groups based on median urinary iodine concentrations (22).

Median Urinary Iodine, mg/L	Iodine Intake	Iodine Nutrition
School-aged children (≥ 6 years) and adults		
< 20	Insufficient	Severe iodine deficiency
20–49	Insufficient	Moderate iodine deficiency
50–99	Insufficient	Mild iodine deficiency
100–199	Adequate	Optimal
200–299	More than adequate	Risk of iodine-induced hyperthyroidism in susceptible groups
> 300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)
Pregnant women		
< 150	Insufficient	
150–249	Adequate	
250–499	More than adequate	
≥ 500	Excessive[a]	
Lactating women and children < 2 years old[b] and children < 2 years old		
< 100	Insufficient	
≥ 100	Adequate	

[a] The term "excessive" means in excess of the amount required to prevent and control iodine deficiency
[b] In lactating women, the median urinary iodine is lower than the iodine requirements (requests) because of the iodine excreted in breast milk.

Most of these techniques are either very expensive or require several stages of manipulation for routine analysis. Methods applied for assessing levels of nutritional iodine in populations, particularly in developing countries, should be rapid, simple, reliable, affordable, and flexible. Presently, urinary iodine determination is performed almost entirely by one of the two methods; an age-old kinetic spectrophotometric method called the Sandell–Kolthoff (S–K) reaction (27, 46) and an unsophisticated method, the so called ICP-MS method which permits superb sensitivity, and as such, sometimes allows direct sample analysis following dilution such as in urine samples (26).

3.2.1. Methods based on the Sandell–Kolthoff reaction

Methods used to determine urinary iodine levels are generally based on the S–K reaction (27). The first step includes elimination of interfering substances and releasing the iodine bound to urine excretory compounds, by chloric acid, ammonium persulfate digestion and ashing digestion. The iodine-releasing step is an essential procedure during urinary iodine digestion. Ashing is not a suitable method for this purpose due to the potential false negative errors (21, 45). The second step involves a reaction between Ce (IV) and As (III) (46). The main disadvantage of this procedure is the need for iodine to be present in order for the catalytic reaction to take place. In addition, substances interfering with the S–K reaction usually affect performance, but chloric acid digestion is an efficient technique for removing them (47). ICCIDD has compiled seven methods based on the S–K reaction, and these are used in several laboratories around the world and of these, chloric acid digestion is one of the most commonly used. Despite that this method provides an accurate measurement, it has several disadvantages (41) including (a) toxic waste production from arsenic trioxide in the S–K reaction (b) acidic gas leakage during sample digestion that requires a special fume hood and (c) difficulty in obtaining chloric acid from chemical vendors because of its instability. The conventional digestion method recommended by Dunn et al. makes use of electrical heat for at least 60 min (41). Sample preparation time to determine urinary iodine levels, can be substantially reduced with the use of rapid microwave digestion and the microplate reading format method (21).

Ammonium per-sulfate digestion has recently been reported as a nonhazardous, non-explosive and easy-to-use procedure but it remains inadequate for testing because it is time consuming and produces a level of toxic waste that exceeds the allowed limitation. Studies have been done to develop an easy-to-use method (48). A microplate format was applied to minimize production of toxic waste and to simplify and speed up the procedure. A closed system was used in the digestion process to keep an equal reaction volume in each well, to avoid contamination and to prevent vapor from leaking and to maximize accuracy in measurements of urinary iodine. This method yields good correlation coefficients when compared to ICPMS and conventional method (chloric acid or ammonium persulphate), has been further developed with a slight modification of the microplate method that makes it amenable to laboratory conditions by using a heating block instead of a cassette (17, 45).

3.2.2. Inductively coupled plasma mass spectrometry (ICP-MS)

In recent years, ICP-MS has become the method of choice for reliable determination of iodine in biological samples. The ICP-MS method is fast, accurate, robust and specific to the evaluation of urinary iodine. Since 2000, in the US's national health and nutrition examination survey (NHANES), urine iodine concentration was determined using ICP-MS (42). Over years, modifications have been made to ICP-MS procedures, mostly at the dilution step or by varying the internal standard (17, 32, 43, 49). In 1998 and 2008, Haldiman et al. (32), and Macours et al. (43), respectively, showed a good correlation between the ICP-MS and S-K method; in the Macours et al. study however the urinary iodine concentration measured by ICP-MS was slightly, but significantly, lower in S–K (43). In general, inter and intra batch coefficients of variation were between 2-5% (17). As a result of specificity, ICP-MS is not only a resource for quality assurance, but it is also particularly adaptable for long-term monitoring of a population's iodine status. Despite the high cost for instrumentation, the application of ICP-MS may soon become a routine procedure in clinical chemistry, mainly because of its ability to measure several trace elements simultaneously. However, this expensive, sophisticated procedure makes the process unrealistic for widespread use. A simple manual method for estimating the prevalence of IDD has recently been proposed (43). The modified methods of the S–K reaction, such as spectrophotometric measurement or microplate reading, are frequently applied in most laboratories. Ammonium persulfate digestion on microplate (APDM) method was compared with the conventional chloric digestion method and the ICP/MS method, and was identified as a sensitive method for urinary iodine detection. There was a good correlation between APDM and the other two methods. No trends or shifts between these methods were detectable in the difference plot (32, 42, 43, 50).

3.3. Iodine determination in milk

The groups of individuals most sensitive to iodine deficiency are pregnant women, lactating women and newborn babies, mainly due to the impact of fetal and neonatal hypothyroxinaemia on brain development (51). Assuming a breast milk intake of 0.78L/day as the only source of iodine for breastfed infants and that single samples represent daily breast milk iodine contents, which may not be accurate because of day-to-day and diurnal

variations in breast milk iodine contents as noted by Kirk et al., 47% of women sampled could have been providing breast milk with insufficient iodine to meet their infant's daily requirement (52). During the first few months of life, breast milk, cow's milk, and formula milk are the exclusive sources of iodine intake in breast-fed, cow's milk–fed, and formula-fed children, respectively. Therefore, milk iodine concentration serves as a useful index to determine infant iodine intake (19). Iodine, a unique trace element, has a high concentration in human breast milk. It is recommended that lactating women increase their dietary intake of iodine to supply the nursing infant with a sufficient supply of the micronutrient. An iodine intake of 250μg/day is recommended for lactating women by WHO/UNICEF/ICCIDD (10) while the Institute of Medicine of the US Academy of Science recommends the higher dose of 290μg/day, whereas Delange suggests that a dose of 225–350μg/day is the appropriate amount (53). Laboratory confirmation of iodine levels is very important to diagnose iodine deficiency and to monitor its treatment. Most iodine in biological samples is covalently bonded and certain substances interfere with the reaction and can affect the results. The mineralization step in milk samples is necessary before analysis; because milk has a high content of lipid that can potentially cause problems in spectrophotometric readings. This is a critical step, as iodine can easily be lost by volatilization during the mineralization step (19, 26). The two main methods used to determine iodine levels are as follows: The instrumental method (54-57), which uses instrumentation such as X-ray fluorescence, polarography, ICPMS, and atomic absorption spectrometry; the second method includes colorimetric methods in association with S-K reaction (26, 27). However, following S-K's report on catalytic reaction methods to determine iodine, methods are now technically very simple and have been used by many laboratories to measure iodine levels for many years (27, 46). The first method (includes high technology–based instrumentation) is very expensive, time consuming, requires expert personnel, additional pre-concentration and separation steps. All methods require processed samples in order to release iodine from organic compounds and removed interference substances. Acid, alkaline digestions and alkaline ashing are three methods commonly used to prepare samples. Sample preparation by ashing requires high temperature, harsh conditions and can produce false-negative results because of iodine volatility. The high lipid content of milk makes it easily dissolvable in hot alkaline media; however, this media is considered very harsh and even dangerous. It is therefore more preferable to use a mild acid media for digestion rather than an alkaline solution. Several methods for acid digestion have been reported for testing iodine levels in milk, however, a long assay period is required and digestion processes are harsh (10, 19, 58). In the Hedayati et al. study a new mild acidic media was used in a quick assay for digestion and to increase sensitivity in reading the results in a microplate reading through a microplate reader (19).

Milk has a low level of iodine, so it is essential to apply a sensitive analytical technique such as ICP-MS. As problems may be encountered during the direct determination of iodine in milk by ICP-MS, a digestion step is necessary. Recently, ICP-MS was employed to determine iodine species in different human milk and infant formulas, wherein iodine bound to casein, whey protein and fat were reported quantitatively (26, 58). The method has the advantage of being applicable to a small sample size; it could however make further fractionation analysis, such as fat fractionation, more difficult. Furthermore, the procedure does not separate inorganic elements from whey proteins, as caseins precipitate while whey proteins remain in the clear part. Thus, the iodine related to the inorganic elements and whey proteins are quantified as a single fraction. Although ICP-MS is undoubtedly a very sensitive technique, and can be used for the quantification of several elements, iodine determination using this technique would however require special care during the digestion step, due to the threat imposed by iodine volatilization (1, 26). The aforementioned methods need either derivatization or digestion step before determination. However, no sample dissolution is required in epithermal instrumental neutron activation analysis (EINAA) of iodine. EINAA method conjugated with Compton suppression spectrometry (EINAA–CS), can be employed to determine iodine with high precision and accuracy without using digestion, and a very low detection limit for the species. It appears that iodine species and fractions in milk are most commonly determined using either ICP or EINAA. In overall, the detection limits for both methods are similar, although NAA can always improve this parameter by extending durations of irradiation and counting (26, 29).

3.4. Iodine determination in serum

TSH (thyroid stimulating hormone) and thyroglobulin (57) serum concentrations can assist as indicators in iodine-deficient areas (Table 3). TSH is a main indicator for iodine deficiency in newborn period due to the high iodine turnover compared to adults. About 1 in 4000 neonates, in iodine sufficient areas, have congenital hypothyroidism and permanent mental damage (59). The proper development of the central nervous system, in particular its myelination is affected by thyroid hormone activity in the perinatal period. For this reason, WHO has established that the results of neonatal blood TSH screening can be used as convenient indicators for iodine intake (10). To detect congenital hypothyroidism and start rapid treatment, dried whole blood spot TSH on filter papers, or occasionally with blood spot T4 tests measured by heel stick from newborns (60). Serum Tg is a sensitive measure of thyroid activity. Serum Tg is elevated in iodine-deficient areas due to TSH hyperstimulation and thyroid hyperplasia. In iodine-deficient infants and children, serum Tg concentrations are often higher than are serum TSH concentrations. Thyroglobulin has also been shown to be a sensitive mea-

sure of excess iodine intake in school-age children that have a median Tg concentration of less than 10 μg/l in a population indicated iodine sufficiency (61 , 62).

In population studies, dried whole blood spots (DBS) on filter paper or serum samples can be used for measuring TSH and/or Tg (60, 62). Normally, a sample of a few drops of whole blood is collected from the cord or by a heel prick from newborns on a filter paper for TSH, or blood spot from school-age children for Tg. Blood can be taken either from the cord at delivery or by heel-prick after birth, usually after 72 hours. Blood spots, once dried, are usually stable for up to six weeks, and can be stored in a plastic bag and transported even through a regular postal service (10). Methods for determining TSH and Tg concentrations from DBS either on filter paper or from serum are well established and widely available. Varieties of commercial kits are available for measuring TSH and Tg which are very sensitive and highly specific competitive radioimmunoradiometric, and immunofluorimetric assays. Most have been carefully standardized and perform adequately. Assays using monoclonal antibodies, which can detect TSH as low as 5mIU/L in whole blood spots, are more often applied to determine iodine deficiency (10, 63).

4. Conclusions

Iodine levels in salt, urine, milk and serum provide information on the key marks used in the assessment of iodine nutritional status in populations. Microplate reading method, which includes the S–K reaction, requires a spectrophotometric microplate reader and is most appropriate for large numbers of samples. The ICP-MS method is reported to yield the most accurate results among technologically advanced methods. The titration method is the universal instrumental method used to quantitatively determine iodine contents in iodized salt, governmental laboratories and academic or research institutions. Rapid test kits offer a qualitative indication for the presence of iodine in salt and are frequently applied in household studies. Potentiometric methods are technologically advanced and accurate; they are also suitable for the analysis of other salt-related variables. However, more research is needed to prove its suitability as an analytical method for the evaluation of salt iodine concentrations. To determine breast-milk iodine concentration, samples must be digested for the release of iodine from organic compounds and interfering substances must first be eliminated. Thereafter, iodine is detected by an analytical technique (ICP-MS) or an S-K reaction. Iodine determination has been performed with high precision and accuracy using EINAA–CS without digestion. This method may be unrealistic for widespread use due to its high cost and degree of sophistication. Dried whole blood spots Tg and TSH are sensitive indicator in iodine deficiency.

Table 3. Indicators of impact at population level: summary

Monitoring Indicator	Age Group for Assessment	Advantages	Disadvantages
TSH, mIU/L	Newborns	Measures thyroid function at a vulnerable age when iodine deficiency directly affects the developing brain	Not recommended to be set up solely to assess community Iodine deficiency due to expense
		If screening programs to detect congenital hypothyroidism are in place then only additional cost will be for data analysis	Cannot be used when antiseptics containing iodine are used during delivery
		Collection by heel stick and storage on filter paper is simple	Requires use of a standardized, sensitive assay
		Blood spots can be stored for several weeks at cool, dry room temperatures	Should be taken either from the cord at delivery or by heel prick at least 48 hours after birth to avoid physiological newborn surge
Tg, μg /L	School-age children	Collection by finger stick and storage on filter paper is simple	Expensive immunoassay
		Can be stored for several weeks at cool, dry room temperatures, so sampling practical even in remote areas	Requires laboratory infrastructure
		Measures improving thyroid function within several months after iodine repletion	
		Standard reference material is now available, but needs to be validated	
		An international reference range has been established	

Acknowledgements

The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication. We would like to thank Ms. Niloofar Shiva for language editing of the manuscript.

Authors' Contribution

The authors have no conflict of interest.

Financial Disclosure

The authors have no conflict of interest.

Funding/Support

This is a review article so no need funding.

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