WHO – ICCIDD – CCM – AIIMS

Second Inter-Country Training Workshop on

Iodine Monitoring, Laboratory Procedures

&

National IDDE Programme

15th – 18th April, 2003

Workshop Venue

Centre for Community Medicine
All India Institute of Medical Sciences
New Delhi 110029, INDIA

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Preface

The workshop to establish a Urinary Iodine Laboratory Network was organized in Bangkok, between 22nd and 25th May 2001, by the Institute of Nutrition, Mahidol University, in association with Centres for Disease Control and Prevention (CDC), Atlanta, World Health Organisation (WHO), United Nations Children's Fund (NICEF) and the Micronutrient Initiative (MI) with other participants being ICCIDD and PAMM. The workshop's mandate was to focus the world attention on not just the setting up of an International Urinary Iodine Laboratory Network, but also the provisions for standardized Internal and External Quality Assurance protocols contained therein.

An outcome of the Bangkok workshop, the ICCIDD South Asia Regional Office, in collaboration with the World Health Organization (WHO), South East Asia Regional Office (SEARO) and All India Institute of Medical Sciences is organizing a Second Inter-Country Workshop on 'Iodine Monitoring Laboratory Procedures and National IDDE Programmes'. In South East Asian region context, the workshop was relevant because there was long felt a need to have a National Network to monitor the iodine content of salt and the urinary iodine excretion. There are many independent laboratories conducting both the salt and urinary iodine analysis, but the system for providing regular exchange of samples between the laboratories was required. One of the important issues to be discussed in the workshop included setting up of standardized internal and external quality control protocols, regardless of the methods of estimation being used. The need for quality epidemiological data in terms of salt iodine and urine iodine depends on laboratory practices and methods that confirm to International standards. With the presence of the trained laboratory managers and other technical professionals in the key centres in the country, and thereby in the region, who can further train others in the sub-national/regional laboratories, the goal of decentralized capacity building will be fulfilled.

The policy issues involved in the establishment of a Urinary Iodine Laboratory Network are multisectoral. The guiding principle in the elimination of IDD in the world should be sustainability, with the integration of the Iterative loop into the social process model ('the Wheel'), to ensure its viability.

As most countries in world are going through a transition phase, the indicators to measure iodine deficiency status have a complex relationship. It is, therefore imperative that there is an availability of representative, reliable data to better track the progress of the programmes through adequate quality control and quality assurance measures. The present workshop is a step forward towards fulfilling the objectives of sustainability.

Preface to the Second Edition

The **Workshop to Establish a Urinary Iodine Laboratory Network** was organized in Bangkok, between 22nd and 25th May 2001, by the Institute of Nutrition, Mahidol University, in association with Centres for Disease Control and Prevention (CDC), Atlanta, World Health Organization (WHO), United Nations Children's Fund (UNICEF) and the Micronutrient Initiative MI) with other participants being ICCIDD and PAMM. The workshops aim was to focus attention on the establishment of a network of International Resource Laboratories for Iodine (IRLI). This workshop was the first in a series of workshops in strengthening the capacity of laboratories around the worked to accurately measure iodine in urine and salt. One of the outcomes of the workshop was the formation of regional, national and sub-national networks to serve as resource Centres for their respective areas. These networks were envisaged to be a part of a technically harmonized network tied to one accuracy base, which would enable the comparability of results across regions and programs.

The structure and early goals International Resource Laboratories for Iodine IIRLI) Network was established at this international conference in Bangkok, which was attended by researchers, policymakers, and public health professionals from 31 countries. The plan of action was to select one or two laboratories from each of the six World Health Organization (WHO) regions on the basis of their laboratory performance, capacity and infrastructure, solid links to a national IDD programming body, and geopolitical representation. The IRLI coordinating body, which includes representatives from the Centers for Disease Control and Prevention (CDC), International Council for the Control of Iodine Deficiency Disorders (ICCIDD), Micronutrient Initiative (MI)(, United Nations Children's Fund (UNICEF), and WHO, then selected 12 laboratories as resource laboratories of the initial IRLI Network.

Twenty-eight representatives from the 12 IRLI resource laboratories and from the co-sponsoring agencies convened in Cape Town, South Africa, for the IRLI Harmonization Workshop during November 10-14, 2002. The workshop was co-sponsored by the CDC, ICCIDD, MI, UNICEF, and WHO, and hosted by Dr. Pieter Jooste and his colleagues of the Medical Research Council, Tygerberg, South Africa. The goals of the workshop were to harmonize the operating procedures among IRLI laboratories, equip IRLI laboratories for their role as resource laboratories, improve communications among laboratories and with the coordinating body, plan next steps to implement network activities at the regional level, and develop long-range plans of action for the six regions.

Merely two months after announcing the designation of IRLI resource laboratories, at least two laboratories received additional resources from their governments as recognition and reinforcement of their new regional function.

As a follow up to the Bangkok workshop, the **ICCIDD South East Asia Regional Office**, in collaboration with **World Health Organization South East Asia Regional Office (SEARO)** and the **All India Institute of Medical Sciences** organized a inter-country workshop on 'Iodine Monitoring, Laboratory Procedures and National IDDE Programmes' between 17th – 24th September 2002. The workshop was attended by representatives from **Sri Lanka, Bangladesh and India (Delhi, Hyderabad and Chandigarh)**. In the South East Asian region context, the workshop was relevant because there was long felt a need to have a national network to monitor the iodine content of salt and the urinary iodine excretion. There are many independent laboratories conducting both the salt and urinary iodine analysis, but the system for providing regular exchange of samples between the laboratories was required. One of the important issues that were discussed in the workshop included setting up of standardized internal and external quality control protocols, regardless of the methods of estimation being used.

In continuation of harmonization of training methods and processes, the **ICCIDD South East Asia Regional Office**, in collaboration with the **World Health Organization South East Asia Regional Office (SEARO)** and the **All India Institute of Medical Sciences** organized a inter-country workshop on 'Iodine Monitoring, Laboratory Procedures and National IDDE Programmes' between 15th – 18th April 2003.

The need for quality epidemiological data in terms of salt iodine and urinary iodine depends on the laboratory practices and methods that conform to international standards. With the presence of the trained laboratory managers and other technical professionals in the key centres in the country, and thereby in the region, who can further train others in the sub-national/regional laboratories, the goal of decentralized capacity building will be fulfilled.

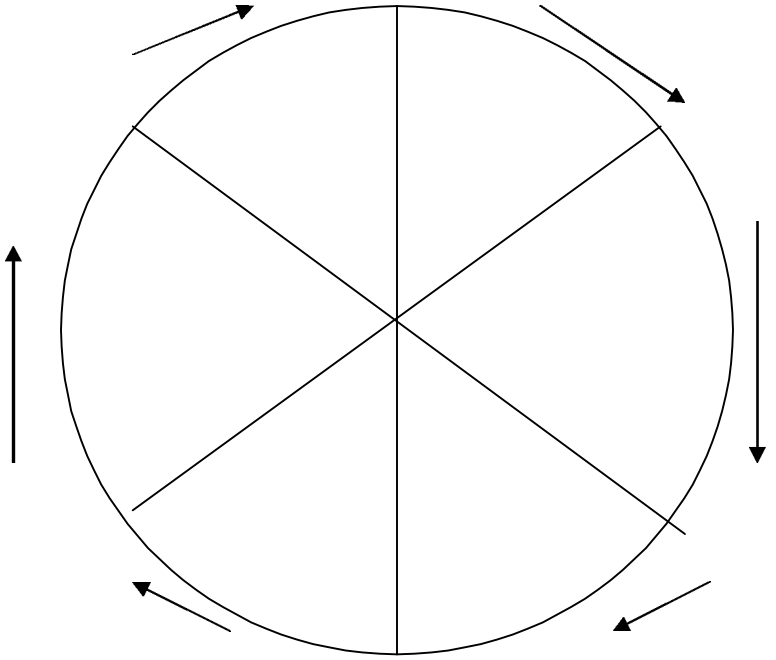
The policy issues involved in the establishment of a urinary iodine laboratory network are multisectoral. The guiding principle in the elimination of IDD in the world should be sustainability, with the integration of the Iterative loop into the social process model ("The Wheel"), to ensure its viability.

The commitment and contribution toward strengthening the capacity of individual country laboratories to accurately measure iodine in urine and salt is vital to ensuring achievement of the goal of sustainable global elimination of IDD by 2005.

Social Process Model for Health Care Programmes (The Wheel)

The Social Process model ("The Wheel") below describes the iterative cycle of IDD elimination. They key lies in the sustainability of IDD elimination, not just its short-term elimination.

TRACKING PROGRESS	POPULATION RISK
IDD Prevalence Urinary iodine Excretion Salt iodine	IDD Prevalence excretion Salt economy



List of Abbreviations

AIIMS	:	All India Institute of Medical Sciences
DIT	:	Diiodotyrosine
EIP	:	Extended Programme for Immunization
ICCIDD	:	International Council for Control of Iodine Deficiency Disorders
IDD	:	Iodine Deficiency Disorders
MI	:	The Micronutrient Initiative
MIT	:	Monoiodotyrosine
NIDDCP	:	National Iodine Deficiency Disorders Control Programme
NIN	:	National Institute of Nutrition
PAMM	:	Programme Against Macronutrient Malnutrition
PPS	:	Probability Proportionate to Size
Tg	:	Thyroglobulin
T_3	:	Triiodothyronine
T_4	:	Thyroxine
TRH	:	Thyrotropin Releasing Hormone
TSH	:	Thyroid Stimulating Hormone
UN	:	United Nations
UNICEF	:	United Nations International Children's Emergency Fund
USI	:	Universal Salt Iodization
WWHO	:	World Health Organization

Contents

1. Iodine & Thyroid Hormone

1.1) Role of Iodine

Iodine is one of the essential micronutrient required for the normal mental and physical well being of human beings. The healthy human adult body contains 15-20 mg of iodine of which 70-80 percent is in the thyroid gland, which weighs only 15-25 gm. The requirement of iodine is 150 micrograms per person per day. This works out to an amount less than a teaspoonful over a life span of 70 years. The tiny quantity of iodine is required everyday by the thyroid gland for adequate production of the hormone thyroxine. It requires four atoms of iodine to make one molecule of thyroxine. It is against this backdrop that a Joint Consultation of the WHO, UNICEF and the International Council for Control of Iodine Deficiency Disorders (ICCIDD) made recommendations of optimal iodine intake in the various age groups **(Table 2).**

1.2) Thyroid Gland: Anatomical Consideration

Thyroid tissue is present in all vertebrates. In mammals, the thyroid originates from an evagination of the floor of the pharynx, and a thyroglossal duct marking the path of the thyroid from the tongue to the neck sometimes persists in the adult. The butterfly shaped gland is situated in the lower part of the front of the neck. The two lobes of the human thyroid are connected by a bridge of tissue, the thyroid isthmus, and there is sometimes a pyramidal lobe arising from the isthmus in front of the larynx. The gland is well vascularized, and the thyroid has one of the highest rates of blood flow per gram of tissue of any organ in the body.

They thyroid is made up of multiple acini (follicles). Each spherical follicle is surrounded by a single layer of cells and filled with pink-staining proteinaceous material called colloid. When the gland is inactive, the colloid is abundant, the follicles are large, and the cells lining them are flat. When the gland is active, the follicles are small, the cells are cuboid or columnar, and the edge of the colloid is scalloped, forming many small “reabsorption lacunae”.

1.3) Iodine Metabolism & Thyroid Hormone Synthesis (Figure 1)

A. Iodide (I, chemically bound iodine) is rapidly absorbed through the gut. The normal intake is 100-150 microgram per day. Iodine is then excreted by the kidney. The level of excretion correlates well with the level of intake. Thus, urinary iodine can be used to assess the level of iodine intake.

B. Concentration of Iodide (I): The thyroid, along with several other epithelial tissues including mammary gland, chorion, salivary gland, and stomach, is able to concentrate I against a strong electro-chemical gradient. This is an

energy-dependent process and is linked to the ATPase-dependent Na^+ - K^+ pump. The activity of the thyroidal I Transporter can be isolated from subsequent steps in hormone biosynthesis by inhibiting organification of I with drugs of the thiourea class (**Figure 1**). The ratio of I in thyroid to iodide in serum (T:S ratio) is a reflection of the activity of this transporter. This activity is primarily controlled by the TSH hormone. The T:S ratio in humans on a normal iodine diet is about 25:1. A very small amount of iodide also enters the thyroid by diffusion. Any intracellular I that is not incorporated into MIT or DIT (generally <10%) is free to leave by this mechanism.

The I transporter is inhibited by two classes of molecules. The first group consists of perchlorate (ClO_4), perrhenate (ReO_4), and pertechnetate (TcO_4), all anions with a similar partial specific volume to I. These anions compete with I for its carrier and are concentrated by the thyroid. A radioisotope of TcO_4 is commonly used to study iodide transport in humans. The linear anion thiocyanate (SCN), an example of the second class, is a competitive inhibitor of I transport but is not concentrated by the thyroid.

C. Oxidation of I: The thyroid is the only tissue that can oxidize I- to a higher valence state, an obligatory step in I-organification and thyroid hormone biosynthesis. This step involves a heme-containing peroxidase and occurs at the luminal surface of the follicular cell.

D. Iodination of Tyrosine: Oxidized I-reacts with the tyrosyl residues in thyroglobulin in a reaction that probably also involves thyroperoxidase. The 3 position of the aromatic ring is iodinated first and the the 5 position to from MIT and DIT, respectively. Once iodination occurs, the iodine does not readily leave the thyroid. Free tyrosine can be iodinated, but it is not incorporated into proteins, since no RNA recognizes iodinated tyrosine.

E. Coupling of Iodotyrosyls: The coupling of two DIT molecules to form T_4 or of an MIT and DIT to form T_3 occurs within the thyroglobulin molecule, although the addition of a free MIT or DIT to a bound DIT has not been conclusively excluded. A separate coupling enzyme has not been found, and since this is an oxidative process, it is assumed that the thyroperoxidase catalyzes this reaction by stimulating free radical formation of iodotyrosine.

This hypothesis is supported by the observation that the same drugs which inhibit I oxidation also inhibit coupling. The formed thyroid hormones remain as integral parts of Tg until the latter is degraded, as described above. Tg hydrolysis is stimulated by TSH, but is inhibited by I, this latter effect is occasionally exploited by using potassium iodide to treat hyperthyroidism.

1.4) Regulation of Thyroid Hormone Synthesis

The regulation of thyroid hormones is a complex process involving not only the thyroid, but the pituitary, the brain, and the peripheral tissues **(Figure 2)**. Thyroid secretion is under the control of the pituitary gland through the thyroid stimulating hormone (TSH). TSH is a glycoprotein with a molecular weight of approximately 28,000. It has two subunits: the 'X subunit' has virtually the same structure as other pituitary hormones, while the ß subunit to specific for TSH, but essentially the same across the different animal species.

The action of TSH occurs through the binding of the ß subunit to specific receptor sites in the membranes of the thyroid cells. The ensuing sequence of events involves the activation of adenylate cyclase which in turn activates the phosphorylation of various proteins and enzymes within each cell including the nucleus, where one major effect is on gene structures for RNA replication and protein synthesis.

TSH activates all stages of iodine metabolism in the thyroid from trapping to the secretion of T_4 and T_3 I transport is accelerated, there is an increase in the organic binding of iodide in the tyrosine molecules, there is an increase in the coupling rate of MIT and DIT to form T_4 and T_3, and then the pinocytosis of colloid by the thyroid cell leads to the release of T_4 and T_3 into the circulation.

The control of TSH secretion is by a "feed-back" mechanism related closely to the level of T_4 in the blood, as the blood T_4 falls, the pituitary TSH secretion rises to increase thyroid activity and the output of T_4 into the circulation, and so maintain the necessary level of circulating hormone. If this is not possible, due for example to severe iodine deficiency, then the level of T_4 remains lowered and the level of TSH remains elevated. Both these measurements are used for the diagnosis of hypothyroidism at various stages in life, but particularly in the neonate.

TSH secretion is also under the control of the brain through the thyrotropin releasing hormone (TRH), which is released from the hypothalamus. TRH is released into the pituitary portal system from where it goes direct to the pituitary. There, if influences the synthesis and release of TSH which is produced by special cells in the anterior lobe of the pituitary **(Figure 2)**. TRH is in turn under the influence of neurotransmitters. Adrenalin, noradrenalin, and serotonin increase its output, and dopamine, which reduce it.

1,5) Physiologic Effects of Thyroid Hormones

The physiologic effects of the thyroid hormones are summarized in **Table 1**.

Table 1: Physiologic effects of Thyroid Hormones[1]

Target tissue	Effect	Mechanism
Nervous system	Developmental	• Promote normal brain development
Bone	Developmental	• Promote normal growth and skeletal development
Heart	Chronotropic	• Increase number and affinity of ß-adrenergic receptors
	Inotropic	• Enhance responses to circulating catecholamines • Increase proportion of Ü myosin heavy chain (with ATPase activity)
Adipose tissue	Catabolic	• Stimulate lipolysis
Muscle	Catabolic	• Increase protein breakdown
Gut	Metabolic	• Increase rate of carbohydrate absorption
Lipoprotein	Metabolic	• Stimulate formation of LDL receptors
Other	Calorigenic	• Stimulate oxygen consumption by metabolically active tissues (exceptions are tests, uterus, lymph nodes, spleen, anterior pituitary) • Increase metabolic rate

2. Iodine Deficiency – Mechanism

2.1) Iodine Deficiency: A Disease of the Soil

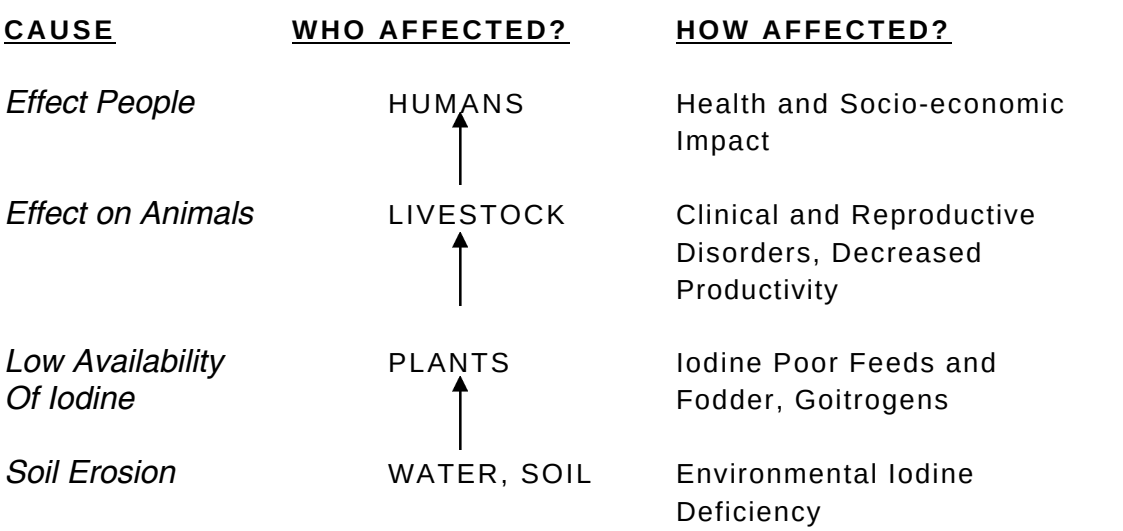
The causes for iodine deficiency in humans should be considered in light of the iodine requirements in human beings. The deal iodine intake as recommended by WHO/INICEF/ICCIDD is shown in **Table 2**.

Table 2: Recommended daily Iodine intake in Human (WHO/UNICEF/ICCIDD)[2]

S.No.	Age Group	Iodine Requirement (in µg)
1.	Infants (0-11 months)	50
2.	Children(12 months to 59 months)	90
3.	School age children (6-12 years)	120
4.	Adults (Above 12 years)	150
5.	Pregnant and lactating women	200

Iodine deficiency occurs when iodine intake falls below recommended levels. Iodine deficiency is an ecological phenomenon occurring naturally in many parts of the world. **Figure 3** depicts the effect of the environmental iodine deficiency on humans, who are perched on top of the food chain.

Figure 3: Iodine Deficiency: A Disease of the Soil



Iodine deficiency is basically a disease of the soil[3] . The atmosphere absorbs iodine from the sea which then returns through the rain and snow to the mountainous regions. It is then carried to the lowers hills and plains, eventually returning to the sea (the Iodine Cycle in nature). The erosion of soils in riverine areas due to loss of vegetation from clearing for agricultural production, overgrazing by livestock and tree-cutting for firewood, ensures a continued and increasing loss of iodine from the soil. High rainfall and snow as well as flood further increase the loss of iodine from the soil which has already been denuded.

The return of the iodine is slow and small in amount compared to the original loss. Groundwater and locally grown plants in these areas also lack iodine. Consequently, the animals feeding on these plants also lack iodine. This results in iodine deficiency in human beings who are dependent on these animals and plants for their dietary supply of iodine. In effect, iodine deficiency is “a disease of the soil” where the environmental deficiency results in the manifestation of the disease state in humans, perched on the top most level of the food chain.

3. Spectrum of IDD & its Consequences

3.1) Spectrum of Iodine Deficiency Disorders

When iodine intake falls below the recommended levels, the thyroid gland is no longer able to synthesis sufficient amount of thyroid hormones. The resulting low level of the thyroid hormones in the blood (hypothyroidism) is the principal factor responsible for an abnormal swelling in the neck called goiter, for the damage done to the developing brain and for many other harmful effects, now described collectively as the Iodine Deficiency Disorders (IDD).[4]

Lack of iodine causes irreparable damage even before childbirth. Children of iodine deficient mothers suffer from brain damage while still in the womb. Thyroxine plays a very important role in the development of the foetal brain and body, in addition to its role in the generation a d utilization of body energy. The optimal development of the human brain from early embryonic to the adult stage is dependent on thyroxine. The most critical period is from the second trimester of pregnancy to the third year after birth. Since 90% of the human brain growth and development is completed by the third birthday, iodine sand subsequent thyroxine deficiency during the early stage of life leads to permanent and irreversible damages. This fact also emphasizes the need for the prevention of iodine deficiency. Many children may even be stillborn. Those who survive can be permanently crippled with a spectrum of mental handicaps and physical deformity – commonly referred to as endemic cretinism.[4]

A less obvious, but more serious condition that handicaps millions of children in iodine deficient areas is damage to the brain and its development in early life. These children suffer from tardy concentration, impaired co-ordination and sluggishness, which result in poor school performances. In addition, their energy and productivity are also adversely affected. It has been estimated that, on an average, school children living in iodine deficient areas have about 12 IQ points lower than the children living in iodine sufficient areas.[5] Thus, the total loss accumulated to the country is formidable.

The term Iodine Deficiency Disorders (IDD) covers a myriad of consequences of iodine deficiency including goitre, cretinism, and its effects on growth, development and even fetal loss. Iodine deficiency is the most common preventable cause of mental handicaps in the world today. The ill effects of iodine deficiency which manifest differently in various stages of life: fetus, neonate, childhood, adolescence and adulthood are given in **Table 3**. Some of the consequences of iodine deficiency are depicted in **Figure 4**.

These disorders can be classified into two categories – those that are irreversible and those that are reversible. The irreversible manifestations are stillbirths, abortions and endemic cretinism (a syndrome consisting of mental retardation, defects in speech, hearing and stunted growth). The reversible manifestations of iodine deficiency include goitre and hypothyroidism.

Table 3: The Spectrum of Iodine Deficiency Disorders[6]

Fetus	• Abortions • Stillbirths • Congenital Anomalies • Increased Perinatal Mortality • Increased Infanty Mortality • Neurological Cretinism: mental deficiency deaf-mutism spastic diplegia squint - Myxedematous cretinism: Mental deficiency Dwarfism - Psychomotor defrcts - Hypothyroidism
Neonate	- Neonatal Goitre - Neonatal Hypothyroidism
Child and Adolescent	- Goitre - Juvenile Hypothyroidism - Impaired Mental Function - Retarded Physical Development
Adult	- Goitre and its complications - Hypothyroidism - Impaired Mental Function - Iodine Induced Hyperthyroidism (IIH)
All Ages	- Increased Susceptibility to Nuclear Radiation

3.2) Iodine Deficiency Disorders in Livestock

Livestock are equally at risk to the spectrum of Iodine Deficiency Disorders at all stages of growth and development, from conception to the adult physical performance. Reproductive failure is the outstanding manifestation of iodine deficiency in livestock. Fetal development may be arrested at any stage leading to death and resorption, abortion and stillbirth, or the birth of young ones that are weak, often associated with prolonged gestation and retention of fetal

membranes. More “kids” are born to iodine sufficient goats and the probability of survival of the “kids” born to iodine sufficient goats is twice that of those born to iodine deficient goats. This is shown in **Table 4**. In addition to the reproductive disturbances described, infertility, both in male and female, has been associated with goitre and this has shown to respond to iodine therapy in iodine deficient areas.

Table 4: Influence of Iodine Supply on the Reproduction of Goats[7]

Description	**Iodine Content (mg/kg) dry substance**	
	0.04 (N=19)	**0.40 (N=18)**
Success of conception (%)	79	83
Success of first insemination (%)	27	73
Abortion rate (%) of gravid goats	47	0
Length of gravidity (days)	158	152
Kids per gravid goat	1.4	1.7
Kids carried to terms per gravid goat	0.9	1.7

4) Epidemiology: Global, Regional and India

4.1) Iodine Deficiency Disorders: A Global Problem

Iodine Deficiency Disorders (IDD) is a major global public health problem. As per the estimates of the WHO (1999), 130 out of the 191 Members States are affected by IDD. Of the remaining 61 countries, there are insufficient data from 41 countries. IDD has been eliminated or is known not to be present in the remaining 20 countries. The details are given in **Table 5**.

Table 5: Countries Affected by Iodine Deficiency Disorders by WHO Region

WHO Region	Total Number of Countries in the Region	Number of Countries		
		IDD is a Public Health Problem	IDD Eliminated* or No IDD	Insufficient Data**
Africa	46	44	1	1
Americas	22	17	3	13
Eastern Mediterranean	22	17	1	4
Europe	51	32	13	6
South East Asia	10	9	0	1
Western Pacific	27	9	2	16
TOTAL	**191**	**130**	**20**	**41**

* IDD Elimination defined as <5% TGR in school aged children

** Insufficient data to categorize countries in either 'IDD remains a Public Health Problem or eliminated'?

Table 6: Endemic Goitre Prevalence by WHO Region*

WHO Region	Population** (In Million)	Population affected by goitre	
		In Million	% of the Region
Africa	612	124	20%
America	788	39	5%
South East Asia	1,477	172	12%
Eastern Mediterranean	473	152	32%
Europe	869	130	15%
Western Pacific	1,639	124	8%
TOTAL	**5,858**	**741**	**13%**

* WHO Global IDD Database (to be published)
** Based on United Nations Population division (UN estimates 1997)

Out of total global population of 5,858 million, an estimated 741 million people are affected by goitre. This comprises about 13% of the total world population. The endemic goitre prevalence by WHO Regions is given in **Table 6**. The highest proportion is contributed by the South East Asian region.

The status of the consumption of iodised salt in the different WHO regions is given in **Table 7**. A total of 68% of the households globally have access to iodised salt, the achievement is a singular feat in itself. The consumption of iodised salt is highest in America – 90%. This is due to the accelerated pace of the programme in the countries in this region, most notably the countries of the sub-Andean belt. It is to be noted that 70% of households in South East Asia and 76% of households in the Western Pacific region consume iodised salt. With one third of the world population residing in these two regions, it is a laudable achievement.

Table 7: Current Status of Household Consumption of Iodised Salt by WHO Region[8]

WHO Region	No. of Countries with IDD	% of Households Consuming Iodised Salt*	
		No data	Overall
Africa	44	8	63%
Americas	19	0	90%
Eastern Mediterranean	17	5	66%
Europe	32	10	27%
South East Asia	9	0	70%
Western Pacific	9	0	76%
TOTAL	**130**	**23**	**68%**

* Total population of each country multiplied by the % of households with access to iodised salt.

Numbers then totaled for each Region and divided by the Total Regional Population.

Table 8: Current Status of Key Elements of IDD Control Programmes

WHO Region	Number of Countries		Number of IDD Affected Countries		
	Affected by IDD	Legislation in Place	Monitoring Salt Quality	Monitoring Iodine Status	With Laboratory Facilities**
Africa	44	34(6*)	29	24	28
Americas	19	17	19	19	19
Eastern Mediterranean	17	14	14	10	11
Europe	32	20(3*)	17	13	13
South East Asia	9	7(1*)	8	7	6
Western Pacific	9	6(2*)	8	6	7
Total	**130**	**98(12*)**	**95**	**79**	**84**
Percent	**100%**	**75% (90%)**	**73%**	**61%**	**65%**

* The figure in brackets refers to the number of additional countries, which have legislation in draft form

**These figures reflect countries with both the capacity for urinary iodine analysis and/or salt iodine levels. The standard of laboratory and expertise for each of these however is very different.

Among the main elements of a successful IDD elimination programme are the establishment of a monitoring system and the provision for legislation to ensure universal coverage of adequately iodised salt. **Table 8** depicts the status of these two elements in the programs all over the world. With 75% of the countries having relevant legislation, 61% monitoring the biological iodine status, 65% having provisions for laboratory facilities, including urine iodine excretion estimation and/or salt iodine estimation, and 73% monitoring salt iodine levels; one can say that tremendous progress have been made in the elimination of IDD. The critical aspect is the consolidation and sustainability of this progress.

4.2) Iodine Deficiency Disorders: South East Asia Region

Iodine Deficiency Disorders (IDD) is a public health problem in many countries of the World Health Organization and in the South East Asian Region (WHO-SEAR) **(Table 9).**

Table 9: Status of IDD Elimination Programmes in WHO-SEAR Countries

No.	Country	Year	Goitre Prevalence	Current Intervention Strategy for IDD Elimination Programme
1.	Bangladesh	1999	17.8%	Iodised salt
2.	Bhutan	1996	14%	Iodized salt
3.	DPR Korea	1996	14%	Iodised salt
4.	Indonesia	1993	33%	Iodised salt, Iodised oil capsule
5.	India	1996	2.3%-68.8%	Iodised salt
6.	Maldives q	1995	24%	Iodised salt introduced
7.	Myanmar	1994	33%	Iodised salt/Iodised oil
8.	Nepal	1999	40%	Iodised salt/Iodised oil
9.	Sri Lanka	2001	18.5%	Iodised salt
10.	Thailand	1998	2.6%	Iodised salt, Water/Iodised oil capsule

The prevalence in India shows a wide range from 2.3% to 68% based on country-wide surveys, and from different States and Union Territories. Besides this, a high prevalence in this region is reported from Nepal where goitre prevalence is 40%. Bangladesh has successfully controlled the problem, bringing down the prevalence rate from 47% in 1993 to 17.8% in 1999. However, it still

remains a public health problem and continued efforts are required to completely eliminate it. Thailand and Bhutan have eliminated IDD as a public health problem. The prevalence of IDD is reduced from 8% in 1994 to 2.6% in 1988 in Thailand. In Bhutan, it has been reduced to 14% in 1996 from 60% in 1983. Thailand, Bangladesh and Bhutan have achieved near universal supply of iodised salt, but due to various reasons, they have not been able to achieve universal availability of iodised salt at the household level.

Table 10 shows the recommended levels of salt iodisation in the countries of the South East Asia region. The availability of iodised salt at the household level in this region ranges from 8% to 82%.

Table 10: Current Status of IDD Elimination Programmes

No.	WHO-SEAR Countries	Recommended iodine content of salt in PPM P=production C=Consumer	Estimated production/ availability of iodised salt as a proportion of total requirement	Estimated proportion of households consuming adequately iodised salt
1.	Bangladesh	P = 50 C = 15	99.7%	57% (1999)
2.	Bhutan	P = 60 C = 30-50	100%	82% (1996)
3.	DPR Korea	P = 30 C = 15	I.A.*	I.A.*
4.	Indonesia	P = 30-80 C = 30	85%	58% (1996)
5.	India	P = 30 C = 15	92% (2000)	71% (1998)**
6.	Maldives	P = Not yet C = Not yet	I.A.*	8% (1995)
7.	Myanmar	P = I.A.* C = I.A.*	I.A.*	I.A.*
8.	Nepal	P = 50 C = 25	87%	55% (1999)
9.	Sri Lanka	P = >50 C = 25	63%	48% (1996)
10.	Thailand	P = 30-50 C = 15	100%	58% (1993)

- I.A. = Information Awaited

**By use of Salt Testing Kits

4.3) Magnitude of the Problem in India

India is the second most populous country in the world with a population of 1027 million (2001 Census). There is a high prevalence of goiter and cretinism in the Himalayan and sub-Himalayan goiter belt from Jammu and Kashmir in the West to Arunachal Pradesh in the East and along this entire length extending at least 500 kms south of the Himalayas into the flat sub-Himalayan *terai* (plans). The Himalayan goiter belt includes Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Uttar Pradesh, Bihar, West Bengal, Sikkim, Assam, Mizoram, Meghalaya, Tripura, Manipur, Nagaland and Arunachal Pradesh[9] .

Table 11: IDD Program Status in India*

State or Union Territory	Total Districts	Districts Surveyed	Endemic Districts	Ban Status	IDD Cell
Andhra Pradesh	23	10	9	Partial	Yes
Arunachal Pradesh	10	10	10	Complete	Yes
Assam	23	18	18	Complete	Yes
Bihar	37	13	13	Complete	Yes
Chhatisgarh	16	2	2	Complete	No
Goa	2	2	2	Complete	Yes
Gujarat	25	16	8	No Ban	Yes
Haryana	19	10	9	Complete	Yes
Himachal Pradesh	12	10	10	Complete	Yes
Jammu & Kashmir	15	14	11	Complete	Yes
Jharkhand	18	9	8	Complete	No
Karnataka	27	17	6	Complete	Yes
Kerala	14	14	11	No Ban	Yes
Madhya Pradesh	45	14	14	Complete	Yes
Maharashtra	35	29	21	Partial	Yes
Manipur	9	8	8	Complete	Yes
Meghalaya	7	2	2	Complete	Yes
Mizoram	8	4	4	Complete	Yes
Nagaland	8	7	7	Complete	Yes
Orissa	30	4	4	Complete	Yes
Punjab	17	3	3	Complete	Yes
Rajasthan	31	3	3	Complete	Yes
Sikkim	4	4	4	Complete	Yes
Tamil Nadu	29	12	12	Complete	Yes
Tripura	4	3	3	Complete	Yes
Uttar Pradesh	71	25	20	Complete	Yes
Uttaranchal	13	9	9	Complete	Yes
West Bengal	18	5	5	Complete	Yes
A & N Island	2	2	2	Complete	Yes
State or Union	**Total**	**Districts**	**Endemic**	**Ban**	**IDD**

Territory	Districts	Surveyed	Districts	Status	Cell
Chandigarh	1	1	1	Complete	Yes
Daman & Diu	1	1	1	Complete	Yes
D & N Haveli	1	1	1	Complete	Yes
NCT Delhi	1	1	1	Complete	Yes
Lakshwadeep	1	1	1	Complete	No
Pondicherry	4	4	4	Complete	No
TOTAL	**587**	**283**	**247**		

*Source: Directorate General of Health Services, Ministry of Health & Family Welfare, 2000.

In addition to the well-known Himalayan endemic belt, iodine deficiency and endemic goiter has been reported from many other States in the county. In 1989, the Indian Council of Medical Research (ICMR) carried out a multi-centric IDD prevalence study. Nine States outside the "traditional goiter-belt" were studied for the prevalence of goiter and cretinism. A total of 409,923 individuals were examined, the overall goitre prevalence observed was 21.1% and the overall cretinism prevalence was 0.7%[10].

Results of sample surveys conducted by different agencies in 283 districts of 29 States and 4 Union Territories of India have shown a high prevalence of IDD in 247 districts. **Table 11** shows the IDD status in the various States and Union Territories. As can be seen, **no State and Union Territory in India is free from IDD as a public health problem**. In 1997, the Government of India introduced a promulgation banning the sale and storage of common salt in the country. All States and Union Territories, with the exception of Kerala (which did not implement the ban at the State level), and Andhra Pradesh and Maharashtra (which implemented the ban partially, i.e. in some district only), implemented the ban order.

The ban order was lifted by the Central Government and in its wake, only Gujarat has revoked the ban order. Other States have maintained at status quo. Under the NIDDCP, there were provisions made for the setting up of **Iodine Deficiency Disorders Cell (IDD Cell)**. The **IDD Cell** at Directorate General of Health Services (DGHS) is responsible for implementation of NIDDCP in the country.

5) IDD Control: Why & How?

5.1) IDD Control: Why?

The effects of iodine supplementation are shown in **Table 12**. The benefits of iodine supplementation in human population are enormous. The consumption of iodised salt by the livestock will also add to the economic productivity of the population. Thus, the cost-benefit ratio of iodine supplementation programmes will increase significantly, making it a worthwhile investment, not only for better human resource development, but will also add to economic contribution from the livestock sector.

Table 12: Effects of Iodine Intervention and Measurements of Benefits

Human Populations	
Effects	**Benefits**
Reduction in: 1. Mental deficiency 2. Deaf-Mutism 3. Spastic diplegia 4. Squint 5. Dwarfism 6. Motor deficiency	1. Value of higher work output in the household and labour market. 2. Reduced costs of medical and custodial care 3. Reduced educational costs from reduced absenteeism and grade repetition and higher academic achievement by students
Livestock Populations	
Effects	**Benefits**
Increase in: 1. Live births 2. Weight 3. Strength 4. Health (less deformity) 5. Wool coats in sheep	1. Value of higher output of meat and other animal products 2. Value of higher animal work output

5.2) IDD Control: How?

5.2a) Survey Methods

5.2a.i) Salt Monitoring

An IDD control programme based on salt iodisation clearly cannot succeed unless all salt for human consumption is being adequately iodised. Therefore the most important thing to monitor is the salt itself, and

the most important place to monitor it is at the site of production. The various sites for monitoring of salt for its iodine content are:

a) Monitoring iodine content at site of production
b) Monitoring iodine content at port of entry
c) Monitoring salt at the point of final packing
d) Monitoring salt at wholesale and retail level
e) Monitoring salt at community level

5.2a.ii) Iodine Status Assessment

Iodine status assessment requires carrying out a cross-sectional survey of a representative sample of the entire target population. The recommended survey method is multistage “probability proportionate to size” (PPS) cluster sampling. This method has been in use for many years for the evaluation of immunization (EPI) coverage, and can be applied to many other health indicators. The target population for the survey should be either school age children or women of childbearing age. Surveys should be either school-based or household based.

5.2a.iii) Sentinel Surveillance

Large scale, representative cross-sectional surveys are generally too costly to be used as a regular instrument regular monitoring of IDD control. To assess the change in iodine status of a defined population over time, the method of monitoring which has proved most practical is the one done through the selection of *sentinel districts*. Such districts are chosen on the basis of their being remote and being affected by moderate or severe IDD prior to the implementation of the IDD control programme. In each sentinel district, at least three rural schools should be chosen at random for surveying. An urban area should also be included to act as a control, and again at least three schools should be selected. Sentinel surveillance surveys should be performed at least every two years in the early stages of an IDD control programme, and then reduced in frequency to once every two or three years once the situation appears stable. It is important to be flexible when establishing a system for monitoring IDD control.

5.2b) Survey Indicators

52b.i) Thyroid Size

Assessment of thyroid size by palpation is the time-honoured method of assessing IDD prevalence, but the long response time after the introduction of iodine supplementation means that it is of limited use in assessing the impact of programme. The term “goitre” refers to a thyroid gland that is enlarged. The

statement that “a thyroid gland each of whose lobes have a volume greater than the terminal phalanges of the thumb of the person examined will be considered goitrous” is empirical, but has been used in most epidemiological studies of endemic goitre. Palpation of the thyroid is particularly useful in assessing the goitre prevalence.

Goitre is graded according to the classification in **Table 13** and this classification is followed in the epidemiological surveys conducted under the National Iodine Deficiency Disorders Control Programme (NIDDCP) in India.

Table 13: Simplified classification of goitre* by palpation

Grade 0	No palpable or visible goitre
Grade 1	A goitre that is palpable but not visible when the neck is in the normal position, even when the thyroid is not visibly enlarged. Thyroid nodules in a thyroid, which is otherwise not enlarged fall into this category.
Grade 2	A swelling in the neck that is clearly visible when the neck is in a normal position and is consistent with an enlarged thyroid when the neck is palpated.

* A thyroid gland will be considered goitrous when each lateral lobe has a volume greater than the terminal phalanx of the thumbs of the subject being examined.

5.2b.ii) Urinary Iodine

Urinary iodine excretion is a good marker of very recent dietary iodine intake as most iodine absorbed in the body eventually appears in the urine. In individuals, urinary iodine excretion can vary somewhat from day to day and even within a given day, but this variation tends to dampen out in populations. The cut-off points proposed for classifying iodine nutrition into different degrees of public health significance are shown in **Table 14**.

Frequency distribution curves are necessary for full interpretation. Urinary iodine values from populations are usually not uniformly distributed, and therefore, the median should be used as the measure of central tendency rather than the mean. (Likewise the percentiles should be used as measures of spread rather than standard deviation). Median urinary iodine concentrations of 100 micrograms per litre (µg/L) and above define a population, which has no iodine deficiency, i.e., at least 50% of the sample should be above 100µg/L. In addition, not more than 20% of samples should be below 50µg/L. Alternatively, the first quintile (20^{th} percentile) should be at least 50µg/L. In adults, a urinary iodine concentration of 100µg/L corresponds roughly to a daily iodine intake of about 150 µg under steady state conditions.

Urinary iodine concentration is currently the most practical biochemical marker for iodine nutrition, when carried out with appropriate technology and sampling. It assesses iodine nutrition at the time of measurement, whereas thyroid size reflects iodine nutrition over months or years. Therefore, populations may have attained iodine sufficiency by median urinary iodine concentration, yet goitre may persist, even in children.

Table 14: Epidemiologic criteria for assessing iodine nutrition based on median urinary iodine concentrations in school-aged children

Median Urinary Iodine (µg/L)	**Iodine intake**	**Iodine nutrition**
< 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 iodine intake	Risk of iodine-induced hyperthyroidism within 5 or 10 years following introduction of iodised salt in susceptible groups
> 300	Excessive iodine intake	Risk of adverse health consequences (iodine-induced hyperthyroidism, auto-immune thyroid diseases)

5.2b.iii) Blood Constituents

Two other indicators are included: thyroid stimulating hormone (TSH) and thyroglobulin (Tg). While TSH levels in neonates are particularly sensitive to iodine deficiency, difficulties in interpretation remain and the cost of implementing a screening programme is too high for most developing countries to afford. The value of thyroglobulin as an indicator of IDD status is yet to be fully explored and it is yet to gain wide acceptance.

5.2c) Indicators for Sustainable Elimination of IDD

Table 15 enlists the criteria for achieving sustainable elimination of iodine deficiency as a public health problem.

Table 15: Summary of criteria for monitoring progress towards sustainable elimination of IDD as a public health problem.

Indicators	Goals*
1. Thyroid size (age group 6-12) Proportion with enlarged thyroid	< 5%
2. Urinary iodine Proportion below 100 µg/L Proportion below 50 µg/L	< 50% <20%
3. Salt iodisation Proportion of households using adequately iodised Salt	> 90%
4. Programmatic indicators Attainment of the indicators listed above	At least 8 out of 10

*The goals are expressed as percentage of population.

- **With regard to the population's iodine status**:

  - The median urinary concentration should be at least 100 µg/L, with less thann20% of values below 50 µg/L.

  - The most recent monitoring data (national or regional) should have been collected in the last 2 years.

- **In iodised salt is the vehicle for the elimination of iodine deficiency, as in almost all countries, there must be guaranteed availability and consumption of adequately iodised salt, demonstrated by more than 90% of households using adequately iodised salt (>15 ppm iodine).Preconditions for this are:**

  - Local production and/or importation of iodised salt in a quantity that is sufficient to satisfy the potential human demand (about 4-5kg/person/year).

  - 95% of the salt for human consumption must be iodised according to Government standards for iodine content, at production or imported level.

  - The percent of food-grade salt with iodine content of at lest 15 ppm, in a representative sample of households, must be equal to or greater than 90%.

  - Iodine estimation at the point of production or importation, and at wholesale and retail levels must be made by titration, while at the household level, it may be made by either titration or certified kits.

- **At least 8 out of the following 10 programmatic conditions are fulfilled**:

  - An effective, functional national body (council or committee) responsible to the Government for the national programme for the elimination of IDD. This council should be multidisciplinary involving the relevant fields of nutrition, medicine, salt industry, education, the media and consumers, with a chair person appointed by the Ministry of Health;

  - Evidence of political commitment to the USI and the elimination of IDD;

  - Appointment of a responsible executive officer for the IDD elimination programme;

  - Legislation or regulations on the USI. While ideally regulations should cover both human and agricultural salt, if the latter is not covered this does not necessarily preclude a country from being certified as IDD-free;

  - Commitment to assessment and re-assessment of progress in the elimination of IDD, with access to laboratories able to provide accurate data on salt and urine iodine;

  - A programme of public education and social mobilization on the importance of IDD and the consumption of iodised salt;

  - Regular data on salt iodine at factory, retail and household level;

  - Regular laboratory data on urine iodine in school aged children with appropriate sampling for higher risk areas;

  - Co-operation from the Salt Industry in maintenance of quality control; and

  - Database with recording of results or regular monitoring procedures, particularly for salt iodine, urine iodine and, if available, neonatal TSH, with mandatory public reporting.

6) Principle and Laboratory Procedures for Iodine Estimation in Salt

6.1 Principle

The iodine content in iodated salt is estimated by a process called iodometric titration. Free iodine reacts with sodium thiosulphate solution as follows:

$2Na_2S_2O_3.5H_2O$ + I_2
Sodium thiosulphate Iodine

= $2NaI$ + $Na_2S_4O_6$ + SH_2O
Sodium iodine Sodium tetrationate

6.2) Equipment and Chemicals

6.2.1) Equipment

1. Laboratory balance for preparing reagents
2. Glass bottles with stoppers for reagents:
   1,000 ml
   250 ml
3. Small weighing scale (One pan balance)
4. Measuring cylinder 50 ml
5. Wash bottle 500 ml
6. Conical flask with stopper 250 ml
7. Glass or plastic funnel
8. Pipette 1 ml or Autodispencer with adjustment of 1ml and 5 ml
9. Pipette 5 ml
10. Burette 10 ml and stand (Auto Burette)
11. A cupboard space to keep the conical flask

6.2.2) Chemicals

1. Sodium thiosulphate – $Na_2S_2O_3.5H_2O$, Analytical Regent Grade (AR).
2. Concentrated sulphuric acid – H2SO4, (AR)
3. Potassium iodide – KI, (AR)
4. Soluble chemical starch
5. Sodium Chloride – NaCl, (AR)

6.3) Preparation of Reagents

a) **Sodium thiosulphate ($Na_2S_2O_3$) 0.005N**: Dissolve 1.24 grams in 1 litre boiled double-distilled water. This volume is sufficient for testing 200 salt samples.Store the solution in a cool and dark place. Normality may change as time progresses Reagents may be prepared fresh in case there is a change in Normality.

b) **2.N Sulphuric acid ($2H_2SO_4$):** To 90ml double-distilled water add 5.56 ml concentrated H_2SO_4 slowly. Add double-distilled water to make 100ml. This volume is sufficient for testing 100 salt samples. Store in a cool dark place. The solution may be kept indefinitely.

Caution: To avoid violent and dangerous reaction always add the acid to water, never water to acid.

c) **Potassium iodide (KI, AR)**: Dissolve 10 grams KI in 100ml double-distilled water. This volume is sufficient for testing 20 salt samples. Store in a cool, dark place. Properly stored, the solution may be kept for 6 months.

d) **Soluble Chemical Starch**: 1% starch solution is prepared in Saturated Sodium Chloride Solution.

Saturated NaCl: Prepare 100ml of saturated salt (NaCl) solution as follows. Dissolve sodium chloride (NaCl) reagent (AR) in 100ml boiled double-distilled water. While stirring, add NaCl unitl no more dissolves. Dissolve 1 gram chemical starches in 10ml boiling double-distilled water. Add the saturated NaCl solution to make 100 ml starch solution. Add Sodium benzoate as a preservative. The solution can be stored for six months.

Table 16: Iodine Content in Parts Per Million (PPM)

Burette reading	Parts Per Million (PPM)	Burette reading	Parts Per Million (PPM)
0.0	0.0	2.5	26.5
0.1	1.1	2.6	27.5
0.2	2.1	2.7	28.6
0.3	3.2	2.8	29.6
0.4	4.2	2.9	30.7
0.5	5.3	3.0	31.7
0.6	6.3	3.1	32.8
0.7	7.4	3.2	33.9
0.8	8.5	3.3	34.9
0.9	9.5	3.4	36.0
1.0	10.6	3.5	37.0

1.1	11.6	3.6	38.1
1.2	12.7	3.7	39.1
1.3	13.8	3.8	40.2
1.4	14.8	3.9	41.3
1.5	15.9	4.0	42.3
1.6	16.9	4.1	43.4
1.7	18.0	4.2	44.4
1.8	19.0	4.3	45.5
1.9	20.1	4.4	46.6
2.0	21.2	4.5	47.6
2.1	22.2	4.6	48.7
2.2	23.3	4.7	49.7
2.3	24.3	4.8	50.8
2.4	25.4	4.9	51.9
5.0	52.9	7.5	79.4
5.1	54.0	7.6	80.6
5.2	55.0	7.7	81.5
5.3	56.1	7.8	82.5
5.4	57.1	7.9	83.6
5.5	58.2	8.0	84.6
5.6	59.2	8.1	85.7
5.7	60.3	8.2	86.8
5.8	61.4	8.3	87.8
5.9	62.4	8.4	88.9
6.0	63.5	8.5	89.9
6.1	64.5	8.6	91.0
6.2	65.6	8.7	92.0
6.3	66.7	8.8	93.1
6.4	67.7	8.9	94.2
6.5	68.8	9.0	95.2
6.6	69.8	9.1	96.3
6.7	70.9	9.2	97.3
6.8	71.9	9.3	98.4
6.9	73.0	9.4	99.5
7.0	74.1	9.5	100.5
7.1	75.1	9.6	101.6
7.2	76.2	9.7	102.6
7.3	77.2	9.8	103.7
7.4	78.3	9.9	104.7

6.5 Precautions

Adding sulphuric acid to a solution of iodated salt liberates iodine, which is titrated with sodium thiosulphate. Starch is used as an external indicator. Potassium iodide solution is added to keep the iodine in the dissolved state.

1. The starch solution must be added near the end of the titration, when very little iodine is left and the solution has a faint-yellow colour. If starch is added earlier, the iodine starch complex becomes very strong and reacts too slowly with sodium thiosulphate, resulting in false high readings.

2. The titration should be done in a comfortably cool room because iodine is volatile and the sensitivity of the starch indicator diminishes as the temperature rises.

3. Potassium iodide (KI) is used because of the low solubility of iodine in water. The liberated iodine forms an unstable complex KI_3 with KI:

$$KI + I_2 = KI_{-3} \text{ and } I + I_2 = I_3$$

As free iodine is used up in the reaction with thiosulphate, the equilibrium between I_2 and I_3 ions is disturbed and more iodine is dissolve in order to maintain the equilibrium.

4. The reaction mixture should be kept in the dark for 10 minutes before titration because light accelerates a side reaction in which iodide ions are oxidized to iodine by atmospheric oxygen.

6.6) Reporting

As you have seen, iodine testing is easy and takes only about twenty minutes per sample. Maintaining accurate records is an important as the testing itself. Record your results in a register, indicating:

- date of testing
- Sample number
- Salt manufacturer's name
- Batch number of the salt
- Where the sample was taken from
- Date of sampling and, finally,
- The level of iodine in the sample.

7. Principle and Laboratory Procedures for Iodine Estimation in Urine

7.1) Principle

Iodine in urine generally occurs as the iodide ion (I). While iodine may be ingested in food or water in a variety of chemical forms, most of it is broken sown to iodide in the gut and absorbed into the blood stream in that form. Virtually all the iodide in the blood either is taken up by the thyroid and converted into thyroid hormone, or is excreted in the urine. The iodide trapped by the thyroid, which may approach 100% of ingested iodine in areas of iodine deficiency, is converted into the thyroid hormones (T_3 and $T_{4)}$ and secreted as hormone into the circulation. In the target tissues, (principally liver, kidney, muscle, and developing brain), iodine is removed from the thyroid hormones and returned to the circulation for eventual excretion by the kidney. Iodine may also escape the body in feces and in breast milk. However, over 90% of the absorbed iodine usually comes out in the urine, presumably as iodide.

Conventional methods for urinary iodine estimation includes an initial step in which the urine is either digested in strong acid or ashed at high temperature. Following that step, iodide is measured by its catalytic action on the reduction of the ceric ion (Ce^{4+}) to the cerous ion (Ce^{3+}) coupled to the oxidation of arsenite, As^{3+}, to As^{5+}. This reaction, called the Sandell-Kolthoff reaction[11], has been diagrammed as follows:

$$2\ Ce^{4+} + 2\ I \longrightarrow 2\ Ce^{3+} + I_2$$

$$I_2 + As^{3+} \longrightarrow As^{5+} + 2\ I$$

The ceric ion (Ce^{4+}) has a yellow color, while the cerous ((Ce^{3+}) is colorless. Thus, the course of the reaction can be followed by the disappearance of yellow color as the ceric ion is reduced. With other reactants held stable, the speed of this color disappearance is directly proportional to the amount of iodide catalyzing it. The reaction can conveniently detect iodide levels down to several nanograms. Because of its specificity and high sensitivity this reaction has been the basis for almost all chemical methods for the detection of iodine in urine.

Excellent discussions of the Sandell-Kolthoff reaction have been published.[12,13] The role of the iodide ion is highly specific; iodate, chloride, and bromide have only slight catalytic activities. Sulfuric acid and chloride are both important components of the reaction mixture; sulfuric acid increases the speed of the reaction, and chloride stabilizes it my inhibiting oxidation of iodide to iodate. Arsenite is present in large excess; some authors have recommended a ratio of (Ce^{4+}) to As^{3+} of 1:20.

Iodide is generally believed to be the chemical form of iodine in the urine and is also the form that is catalytic in the Sandell-Kolthoff reaction. Therefore, it is reasonable to ask why a digestion or ashing step is necessary to prepare urine samples for iodine determination by this method. The answer is that digestion or ashing removes other substances, such as nitrite, thiocyanate or ferrous iron that might interfere by reducing or oxidizing the ceric or arsenite reactants. For example, thiocyanate in the urine can accelerate the Sandell-Kolthoff reaction, thus arti-factually elevating the apparent iodide value[14]. Thiocyanate is derived from the ingestion of cassava which is consumed in many parts of the world, including those with iodine deficiency, and its interference with the Sandell-Kolthoff reaction in undigested urine can introduce serious error in iodine estimation. Cigarette smoking is another frequent source of thiocyanate; heavy smokers (10 or more cigarettes per day) have urinary thiocyanate levels as high as those of cassava eaters. Other interfering substances have not been as well characterized, but most observers have noted that failure to incorporate a digestion or ashing step can arti-factually raise or lower the apparent iodine concentration in the Sandell-Kolthoff reaction, presumably by failing to remove such substances. It has been found that the iodine concentration determined in the undigested sample was about 72% the value found for the same sample when digested. He proposes a correction factor to adjust for this difference.

Garrey et al.[15] described a method using dialysis rather than digestion prior to colorimetric determination of urinary iodine, and found that dialyzed samples gave essentially the same values as digested ones. However, these samples came from children in iodine sufficient areas of the United States, so virtually all samples were above 5µg/dl, and most were above 20µg/dl. May et al.[14] reported that the dialysis technique gave arti-factually high iodine values for urine samples from iodine-deficient areas of China, sometimes doubling the true values obtained after ashing.

We conclude that at present the safest course is to include an ashing or digestion step prior to colorimetric reaction of urinary iodine. However, if one can show that assays of digested and undigested aliquots of the same urine samples consistently give the same iodine value and that this result is homogenous throughout a given study population, then omission of the ashing or digestion step may be feasible.

Other methods have occasionally been employed for urinary iodine determination, but they are generally not practical for survey purposes. Ion specific electrodes can determine iodine in urine, but currently are not sensitive enough to recognize the low levels encountered in areas of iodine deficiency, and determination is not rapid.[16] Neutron activation is sensitive and specific, but cannot be done on large number of samples in a routine manner.[17] Gas liquid chromatography has also been used, but requires special equipment and is not as rapid as some of the methods described here for epidemiologic purposes.

In summary, the Sandell-Kolthoff reaction, with some prior step involving ashing or digestion, is usually the most practical approach for laboratory determination of urinary iodine.

The two methods described in this chapter are (1) ammonium Persulfate Digestion Microplate (APDM) method, and (2) Conventional Ammonium Persulfate Digestion in Test Tune and Spectrophotometer (NIN, Hyderabad) method. These two methods are commonly being used in most of the iodine monitoring laboratories. These methods use ammonium persulfate for digestion and avoid chloric acid which generates toxic fume.

7.2) Equipment and Chemicals

7.2.1) Equipment

a) Equipment needed for both the methods

- Frigidaire to store urine samples
- Reagent flasks and bottles
- Pipettes
- Weighing balance scale

b) Equipment needed for APDM method

- Sealable Heating Cassettes for Microplates
- Flat Bottomed Microplates
- Round Bottomed Microplates
- Microplate Reader
- Micropipetttes
  - Single Channel 10µL – 100µL
  - Single Channel 100µL – 1000µL
  - Multi Channel 20µL – 200µL

- Disposable tips for imcropipettes

c) Equipment need for Spectrophotometer method

- Oven / Heating block
- Colorimeter / Spectrophotometer

7.2.2) **Chemicals** (for both the methods)

1. Potassium iodate (for calibrators)
2. Arsenic trioxide (analytical grade)
3. Ammonium persulfate

4. Tetraammonium cerium (IV) sulfate dehydrate
5. Sodium chloride
6. Sulfuric acid
7. Glass distilled /deionized water

7.3) Preparation of Reagents

a) Ammonium persulfate solution:
APDM method. Ammonium persulfate (30g) was dissolved in water to a final volume of 100mL. This solution was prepared fresh just before use.
Spectrophotometer method. Dissolve 14.1g $H_2N_2O_8S_2$ in H_2O, make up to 500 ml with H_2O. Store away from light. Stable for at least one month.

b) 5 N H_2SO_4: Slowly add 139ml concentrated (36 N)H_2SO_4 to about 700 ml deionized water careful – this generates heat! When cool, adjust with deionized water to final volume of 1 litre.

c) Arsenious acid solution:
APDM method. Arsenic trioxide (5 g) was dissolved in 100mol/L sodium hydroxide solution. Concentrated sulfuric acid (16 mL) was then added slowly to the solution in an ice bath. After cooling, 12.5g of sodium chloride was added to the solution, and the mixture was diluted to 500 mL with cold water and filtered.

Spectrophotometer method. In a 2000ml Erlenmeyer flask, place 20g As_2O_3 and 50g NaCl, then slowlly add 400ml 5 N H_2SO_4. Add water to about 1 litre, heat gently to dissolve, cool to room temperature, dilute with water to 2 litres, filter, store in a dark bottle away from light at room temperature. The solution is stable for months.

d) Ceric ammonium sulfate:
APDM method. Tetraammonium cerium (IV) sulfate dehydrate (6g) was dissolved in 3.5N sulfuric acid and adjusted to a final volume of 500mL with the same acid solution.
Spectrophotometer method. Dissolve 48g ceric ammonium sulfate in 1 litre 3.5 $\underline{N}H_2SO_4$. (The 3.5 $\underline{N}$ H_2SO_4 is made by slowly adding 97 ml concentrated (36 $\underline{N}$) H_2SO_4 to about 800l deionized water (careful – this generates heat!), and when cool, adjusting with deionized water to a final volume of 1 litre). Store in a dark bottle away from light and keep at room temperature. The solution is stable for months.

e) Iodine calibrators: 168.6mg of potassium iodate KIO_3 (equivalent to 100 mg of I_2) is dissolved in 100ml of double distilled/deionized water (1 mg/ml of Iodine). This is the stock solution (solution A).

dilute stock solution 1 to 1000ml to get a solution of 1µg/ml concentration of Iodine solution (solution B).

Prepare from solution B the working standards containing 20,50,100, 150,200,250,300 µg of I_2/litre by diluting appropriately.

7.4) Collection & Storage of Urine Samples:

Iodide in the urine is stable under most circumstances. Urine samples of several milliliters each can be collected in the field and transferred to screw cap plastic or glass bottles that can be tightly closed to avoid spillage. They can be transported without refrigeration. The small volumes allow many samples to be conveniently packaged and brought from the field to the laboratory, or shipped by mail. For long-term storage, urine samples are usually kept in the Refrigerator. Before analyzing, samples should be brought to room temperature.

7.5) Estimation of Iodine Content in Iodated Urine: Laboratory

Procedures

7.5.1) APDM method:

1. Calibrators and urine samples (50µL each) were pipetted into the well of a round bottom polypropylene (PP) plate, followed by the addition of 100µL of ammonium persulfate solution (final concentration, 0.87 mol/L) (30% W/V).

2. The PP plate was set in a cassette. The cassette was tightly closed and was kept for 75 min in an oven adjusted to 100°C.

3. After digestion, the bottom of the cassette was cooled to room temperature with tap water to avoid condensation of vapor on the top of wells and to stop the digestion.

4. The cassette was opened, and 50-µL aliquots of the resulting digest were transferred to the corresponding wells of a polystyrene 96-well microtiter plate (Flat Bottom).

5. Arsenious acid solution (100µL) was added to the wells and mixed; 50µL of ceric ammonium sulfate solution was then added quickly (within 1 min), using a multi channel pipette.

6. The colour change was monitored by measuring absorbance at 405nm with a Microplate reader at 5,10.15,20.25 minutes.

7.5.2) Spectrophotometer method

1. Mix urine to suspend sediment.

2. Pipette 250µl of each urine sample into a 13x100 mm test tube. Pipette each iodine standard into a test tube, and then add H_2O as needed to make a final volume of 250µl. Duplicate iodine standards and a set of internal urine standards should be included in each assay.

3. Add 1ml 1.0M ammonium persulfate to each tube.

4. Heat all tubes for 60 minutes at 100°C.

5. Cool tubes to room temperature.

6. Add 2.5ml arsenious acid solution. Mix by inversion or vortex. Let stand for 15 minutes.

7. Add 300µl of ceric ammonium sulfate solution to each tube (quickly mixing) at 15-30 second intervals between successive tubes. A stop watch should be used for this. With practice, a 15 second interval is convenient.

8. Allow to sit at room temperature. Exactly 30 minutes after addition of ceric ammonium sulfate to the first tube, read its absorbance at 420nm. Read successive tubes at the same interval as when adding the ceric ammonium sulfate.

9. Calculation of results: Construct a standard curve on graph paper by plotting iodine concentration of each standard on the abscissa against its optical density at 405µg/l (OD_{405}) on the ordinate.

Note that

1. This is modified from the former method (see reference below), run blanks and standards with each assay to allow for variations in heating time, etc.

2. Since the digestion procedure has no specific end-point, it is essential to run blanks and standards with each assay to allow for variations in heating time, etc.

3. The exact temperature, heating time, and cooling time may vary. However, within each assay, the interval between the time of addition of ceric ammonium sulfate and the time of the reading must be the same for all samples, standards, and blanks.

4. The cassette was opened, and 50-µL aliquots of the resulting digests were transferred to the corresponding wells of a polystyrene 960well microtiter plate (Flat Bottom)

5. Arsenious acid solution (100µL) was added to the wells and mixed; 50µL of ceric ammonium sulfate solution was then added quickly (within 1 min), using a multi channel pipette.

6. The colour change was monitored by measuring absorbance at 405 nm with a microplate reader at 5,10.15,20.25 minutes.

7.5.2) Spectrophotometer method

1. Mix urine to suspend sediment.

2. Pipette 250µl of each urine sample into a 13x100mm test tube. Pipette each iodine standard into a test tube, and then add H_2O as needed to make a final volume of 250¸l. Duplicate iodine standards and a set of internal urine standards should be included in each assay.

3. Add 1ml 1.0M ammonium persulfate to each tube.

4. Heat all tubes for 40 minutes at 100°C.

5. Coll tubes to room temperature.

6. Add 2.5ml arsenious acid solution. Mix by inversion or vortex. Let stand for 15 minutes.

7. Add 300µl of ceric ammonium sulfate solution to each tube (quickly mixing) at 15-30 second intervals between successive tubes. A stop watch should be used for this. With practice, a 15 second interval is convenient.

8. Allow to sit at room temperature. Exactly 30 minutes after addition of ceric ammonium sulfate to the first tube, read its absorbance at 420nm. Read successive tubes at the same interval as when adding the ceric ammonium sulfate.

9. Calculation of results: Construct a standard curve on graph paper by plotting iodine concentration of each standard on the abscissa against its optical density at 405µg/l (OD_{405}) on the ordinate.

Note that

1. This is modified from the former method (see reference below), substituting ammonium persulfate for chloric acid as digestant.

2. Since the digestion procedure has no specific end-point, it is essential to run blanks and standards with each assay to allow for variations in heating time, etc.

3. The exact temperature, heating time, and cooling time may vary. However, within each assay, the interval between the time of addition of ceric ammonium sulfate and the time of the reading must be the same for all samples, standards, and blanks.

4. With the longer ceric ammonium sulfate incubation and with 15 second interval additions of CAS, up to 120 tubes can be read in a single assay.

5. The volumes and proportions of samples and reagents can be varied to achieve different concentrations or a different curve shape, if conditions warrant. If different tube sizes are used, corresponding sized holes in the heating block are also needed.

6. If necessary, this method could probably be applied without a heating block. Recent observations initiate oven at 100°C is equally suitable. It is essential that all tube, be uniformly heated and that the temperature be constant within the range described above.

7. Test tubes can be reused if they are carefully washed to eliminate any iodine contamination. Care should to avoid mixing of glassware used for preparation of with glassware used for doing iodine estimation in urine.

8. Various steps of this procedure are suitable for automation. For example, the colorimetric readings can be done in micro-titer plates with a scanner, and the standard curves plotted and read on a simple desk computer.

7.6) Precautions

An iodine-free work place: The methods described in this book detect minute amounts of iodine. Any iodine in the environment or in reagents will grossly distort analytic values for urinary iodine. Also, reducing or oxidizing substances in the environment can interfere, for example, the thiocyanate in cigarette smoke. It is difficult and unnecessary to identify all possible contaminants; rather, the technician should exercise meticulous care and cleanliness in all stages of the analysis, including preparation of standards and reagents.

Some important rules:

a. Weigh standard iodate samples well away from the area of subsequent analysis, preferably in a different room. Carefully avoid carrying any iodine back to the analytical area on clothing, spatulas, hands etc. Wash hands carefully after preparing standards.

b. It is best to have a dedicated area or room for iodine analyses in urine. Keep the working area well separated from any other work with iodine, such as making up iodine solutions for iodized water etc. Also, keep the area for iodine analysis well protected from other laboratory procedures with oxidizing or reducing agents, to avoid contamination. It is strictly recommended that analysis of urine and salt should be done in different rooms.

c. The chemical reagents must be iodine-free. Use analytic grade only. Reagents must also be free of moisture. For example, KI is hygroscopic, and if used as a standard, care must be taken in weighing it to avoid moisture accumulation, particularly in hot humid climates. In accordance with general recommended laboratory procedure, do not pipette directly from a reagent bottle, but instead pour into another vessel slightly more than the approximate amount needed, and after pipetting the needed amount, discard the excess rather than returning it to the reagent bottle.

d. The water used for reagents and dilution of samples must be iodine free. Most laboratories will have distilled and/or deionized water. This water should be routinely checked to make sure it is iodine free. Water can be easily deionized by passing through an appropriate resin.

e. Much of the glassware in iodine analyses can be reused, but it must be carefully washed, including a final wash with deionized iodine-free water. Most disposable pipette tips and other plastic items as supplied by the manufacturer are unlikely to have contaminating iodine.

f. Glassware and tips used for preparation for standard iodine solution should be washed and kept separately from other glassware and tips used for carrying out urinary iodine estimation.

g. The technician must scrupulously avoid any opportunity to introduce contaminating iodine. Hands should be washed carefully and frequently. Disposable gloves are useful. Eating, spitting, drinking, and smoking should not take place in the iodine laboratory.

7.7) Reporting

Measurement of urinary iodine level is a good tool for measuring the prevalence of iodine deficiency in a community and the effects of iodised salt. Maintaining

accurate records is as important as the testing itself. Record your results in a register, indicating

- Demographic details
- Where the sample was taken from
- Date of sampling,
- Sample number
- Date of testing, and finally
- The level of iodine in the sample.

Computer for data handling: Computers can be used at most stages of the analysis and expression of results. They can draw standard cures, correct for dilutions, and place results in a data base to interface with other data on the individuals or populations, such as goiter size, blood spot TSH, and more general parameters. The extent of computer application will depend on available computers and technical expertise in their use, and the volume of information to be processed. For small laboratories, much of the initial data handling may be manual, but for larger operations, computers will be essential in handling and storing data on urinary iodine, and relating them to other parameters of iodine deficiency.

Conversion to SI units: Most clinical journals now use SI units for expressing laboratory values. **Tale 18** converts µg/dl to µmol/L, rounded off to the nearest 0.01µmol/L to convert iodine (atomic weight 127), multiply µg/dl by 1/127 (i.e., by 0.079).

Table 17: Conversion to SI units

µg/dl	µmol/L	µg/dl	µmol/L
1	0.08	10	0.79
2	0.16	11	0.87
3	0.24	12	0.95
4	0.32	13	1.03
5	0.40	14	1.11
6	0.47	15	1.18
7	0.55	20	1.57
8	0.63	25	1.97
9	0.71		

8) Quality Assurance in IDD Control Programme

The indicators used to assess the impact of a National IDD Control Programme are total goiter rate, Iodine content of salt, Urinary iodine excretion and TSH. The two most important indicators are laboratory parameters. So it is important that there be a quality assurance mechanism to ensure reliability of the results. Quality assurance is a proactive and continuous process of monitoring a system for reproducibility and reliability by:

a) Setting standards of performance & designating possibility
b) Ensuring definitive corrective actions are taken when the criteria not met
c) Performing measurements within a stated level of confidence.

A quality control system is an indictor system for documented performance and actions that:

a) Provides a record of consistency of performance
b) Records action taken when performance fails to meet standard.

This system uses a non-blinded system with a potential for bias.

For all lab parameters, quality assurance is a must. WHO/UNICEF/ICCIDD has identified the following lab parameters for monitoring IDD Control Programme

Iodine content of salt at production and household level
Urinary iodine levels
Neonatal TSH

There are essentially two types of Quality Assurance protocols:

1) Internal Quality Assurance
2) External Quality Assurance

Internal Quality Assurance

The general guiding principles of intenal quality assurance are:

1. To run one sample with known value with every batch of test sample analysis. Usually 1 known sample should be analyzed with 25-30 unknown samples.

2. Known value sample can be obtained commercially or can be prepared in the laboratory.

3. For urinary iodine laboratory, known values are not available commercially.

4. Known value samples can be prepared in the laboratory.

The key step is the preparation of the known value samples. This step is slightly different for urinary iodine estimation and salt iodine estimation.

Preparation of known value samples for Urinary Iodine Estimation

1. Pooled urine sample or urine collected from one individual.
2. Analyze iodine in the pooled samples for 25 times.
3. Calculate mean $\pm$ S.D.
4. Divided pooled sample in small aliquot of 1ml.
5. Preserve in cold (4-8C).
6. Take out one aliquot each time for internal quality assessment.
7. After using this aliquot, it should be discarded.
8. Aliquots are stable at this temp. for 6 months.

Preparation of known value samples for Salt Iodine Estimation

1. A sample of salt (1/2 – 1kg) collected from the market.
2. Analyze iodine in the salt samples for 25 times.
3. Calculate mean $\pm$ S.D.
4. Divided the collected sample in small sachets of 10 grams each.
5. Preserve at room temperature away from heat and moisture.
6. Take out one sachet of salt each time for internal quality assessment, to be run with the normal samples.
7. The sachets are stale if stored properly, for 6-12 months.

Procedure for Internal Quality Assurance

1. By running the known value sample along with every batch of test sample analysis (25-30 unknown samples).

2. Quality control results can be reported by the system in several different ways. Graphically, data can be presented in Levy-Jennings format.

3. If the value falls between $\pm$ 2 S.D.

   Indicates consistency of method
   Reagents quality
   Performers ability

4. If the value falls outside $\pm$ 2 S.D.

Check aliquots for internal quality
Check reagents for contamination
Pipetting error
Any other

External Quality Assurance

External quality assurance is a process by which the quality of results from each laboratory can be ensured. The known value samples are exchanged between the laboratories participating in the quality assurance program with a central laboratories functioning as a reference laboratory. The reference laboratory is to be recognized by participating laboratories.

The functions of reference laboratory general include:

i) Prepare samples on same lines as internal quality assurance (Mean $\pm$ S.D.).
ii) Send one sample to each participating laboratory twice a month.
iii) Send the computed hart to each participating lab once a month.
iv) Training of laboratory staff at periodic intervals.
v) Providing quality reagents.
vi) Providing external quality assurance samples prepared fresh every time.
vii) Analyzing certain number of samples from each participating laboratory on a regular basis.
viii) Coordination of all laboratories.

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