The Challenge of Iodine Deficiency Disorder
A Decade of CDC's Ensuring the Quality of Urinary Iodine Procedures Program

National Center for Environmental Health
Division of Laboratory Sciences
Acknowledgements

One of the greatest benefits of our program is the high level of confidence that participating laboratories have in the results they obtain while conducting their urinary iodine procedures. This confidence is delivered through the work of many dedicated individuals, whom the authors would like to now recognize.

The authors thank EQUIP collaborators who have provided constructive feedback regarding their experience with the program: Dr. Chandrakant S. Pandav (All India Institute of Medical Sciences), India; Dr. Victor J. Temple (University of Papua New Guinea), Papua New Guinea; Dr. Yuqin Yan (Tianjin Medical University), China; Dr. Kostas Markou and Dr. Irene Mamali (University of Patras Medical School), Greece; Dr. Ludmila Ivanova (United Nations Children's Fund); Dr. Frits van der Haar (Rollins School of Public Health, Emory University), Georgia, USA; and Dr. Arnold Timmer and Dr. Juliawati Untoro (United Nations Children's Fund), New York, USA.

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In addition, we would like to thank our colleagues from CDC's International Micronutrient Malnutrition Prevention and Control (IMMPaCt) Program for their continued contribution to the EQUIP Program.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>APDM</td>
<td>Ammonium Persulfate Digestion Microplate</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>CRM</td>
<td>Certified Reference Materials</td>
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<tr>
<td>EQUIP</td>
<td>Ensuring the Quality of Urinary Iodine Procedures</td>
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<tr>
<td>ICCIDD</td>
<td>International Council for the Control of Iodine Deficiency Disorders</td>
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<tr>
<td>ICP-MS</td>
<td>Inductively Coupled Plasma-Mass Spectrometry</td>
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<td>IDD</td>
<td>Iodine Deficiency Disorders</td>
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<tr>
<td>IQ</td>
<td>Intelligence Quotient</td>
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<td>IRLI</td>
<td>The International Resource Laboratories for Iodine</td>
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<tr>
<td>LOD</td>
<td>Limit of Detection</td>
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<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<tr>
<td>NIST</td>
<td>National Institute of Standards and Technology</td>
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<tr>
<td>PP</td>
<td>Polypropylene</td>
</tr>
<tr>
<td>PPB</td>
<td>Parts Per Billion</td>
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<tr>
<td>PPM</td>
<td>Parts Per Million</td>
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<tr>
<td>PPT</td>
<td>Part Per Trillion</td>
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<tr>
<td>QA</td>
<td>Quality Assurance</td>
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<tr>
<td>QC</td>
<td>Quality Control</td>
</tr>
<tr>
<td>RLT</td>
<td>Range of Linearity Tested</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>S-K</td>
<td>Sandell–Kolthoff Reaction</td>
</tr>
<tr>
<td>STD</td>
<td>Stock Standard Solution</td>
</tr>
<tr>
<td>TMAH</td>
<td>Tetramethylammonium Hydroxide</td>
</tr>
<tr>
<td>UI</td>
<td>Urinary Iodine</td>
</tr>
<tr>
<td>UNICEF</td>
<td>United Nations Children's Fund</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Iodine Deficiency: An Underappreciated Health Risk

Iodine is a micronutrient used by the body to make thyroid hormones, which are necessary for normal growth, development and metabolism throughout life. A billion people—roughly 15% of the world population—suffer from an iodine deficiency disorder (IDD).

While diet-induced hypothyroidism (inadequate production of thyroid hormones) can occur at any stage of life, the most devastating consequences of iodine deficiency take place during fetal development and early childhood. Iodine deficiency during pregnancy can cause miscarriage, stillbirth and congenital abnormalities such as cretinism, a severe and irreversible form of mental retardation. In infancy and childhood, iodine deficiency can retard physical development, give rise to goiter (enlarged thyroid gland) and impair mental function to various degrees.

Iodine deficiency is the leading and most preventable cause of mental retardation in the world. At the population level, the consequence of iodine deficiency is a 10–15% lower average intellectual quotient (IQ), which affects the social and economic development of both communities and nations. There are several strategies available to combat the prevalence of IDD, the most common of which is salt iodization.
Seaweed is discovered to be a source of iodine, and is used extensively for the treatment of goiter, a swelling of the thyroid gland related to iodine deficiency.

Universal salt iodization is first introduced in Switzerland.

The World Health Organization (WHO) presents the first comprehensive review of goiter on a global scale, underlining the severity of IDD.

The concept of IDD is introduced with an emphasis on the effects of iodine deficiency on brain function.

The International Council for the Control of Iodine Deficiency Disorder (ICCIDD) is founded.

The United Nations World Summit for Children, attended by 71 Heads of State and representatives of 15 governments, adopts an action plan that includes the virtual elimination of IDD by the year 2000. This date is later revised to 2005.

Only about 20% of households in the world use iodized salt.

An estimated 70% of households in the world use iodized salt.

CDC’S EQUIP program is established with 27 labs from 20 countries.

The International Resource Laboratories for Iodine (IRLI) Network is established.

More than 120 countries have universal salt iodization programs, and the number of countries where IDD has remained a problem drops to 47.

EQUIP serves more than 120 laboratories in the U.S. and over 60 countries.
How big is the problem?

The World Health Organization (WHO) defines optimal iodine intake—measured as median urinary iodine (UI) concentration—as 100-199 µg/L. Data from the WHO Global Database on Iodine Deficiency indicate that general iodine intake is below this level in at least 47 countries and among an estimated 31.5% of school-age children worldwide.

Data from CDC’s 2007-2008 National Health and Nutrition Examination Survey (NHANES) show that the median UI level for the general US population age 6 and older is 164 µg/L, well within the optimal range. However, 8.8% of the population has UI levels below 50 µg/L (moderate to severe iodine deficiency), including an estimated 14.6% of women of reproductive age. Also 57.5% of women of reproductive age have less than adequate (150 µg/L) UI levels. Non-Hispanic black women of reproductive age tend to have lower UI levels than reproductive-age women of other racial/ethnic groups.

Given the significant numbers of people at risk for IDD worldwide and among select subgroups in the US, population-wide monitoring to document UI levels is a necessary public health activity.
The Challenge of Iodine Deficiency Disorder

EQUIP 10 year anniversary

A plant for refining salt, India
Urine Analysis: The Preferred Way to Measure Iodine Sufficiency

Urinary iodine analysis is the most common method for assessing population-wide iodine sufficiency. Because more than 90% of dietary iodine is excreted in the urine, UI concentrations directly reflect iodine intake. Moreover, accurate laboratory measurement of UI concentrations is readily achievable (Table 1).

<table>
<thead>
<tr>
<th>Median Urinary Iodine (µg/L)</th>
<th>Iodine Intake</th>
<th>Iodine Nutrition</th>
</tr>
</thead>
<tbody>
<tr>
<td>School-Aged Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>Insufficient</td>
<td>Severe iodine deficiency</td>
</tr>
<tr>
<td>20-49</td>
<td>Insufficient</td>
<td>Moderate iodine deficiency</td>
</tr>
<tr>
<td>50-99</td>
<td>Insufficient</td>
<td>Mild iodine deficiency</td>
</tr>
<tr>
<td>100-199</td>
<td>Adequate</td>
<td>Optimal</td>
</tr>
<tr>
<td>200-299</td>
<td>Above Requirement</td>
<td>Likely to provide adequate intake for pregnant/lactating women but may pose a slight risk in the overall population</td>
</tr>
<tr>
<td>&gt;300</td>
<td>Excessive*</td>
<td>Risk of adverse health consequences: (iodine-induced hyperthyroidism, autoimmune thyroid disease)</td>
</tr>
<tr>
<td>Pregnant Women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;150</td>
<td>Insufficient</td>
<td></td>
</tr>
<tr>
<td>150-249</td>
<td>Adequate</td>
<td></td>
</tr>
<tr>
<td>250-499</td>
<td>More than adequate</td>
<td></td>
</tr>
<tr>
<td>≥500</td>
<td>Excessive</td>
<td></td>
</tr>
<tr>
<td>Lactating Women**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>Insufficient</td>
<td></td>
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<tr>
<td>≥100</td>
<td>Adequate</td>
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<tr>
<td>Children Less Than 2yr Old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>Insufficient</td>
<td></td>
</tr>
<tr>
<td>≥100</td>
<td>Adequate</td>
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</tbody>
</table>

* The term “excessive” means in excess of the amount required to prevent and control iodine deficiency.

** In lactating women, the figures for median urinary iodine are lower than the iodine requirements because of the iodine excreted in breast milk.
EQUIP: Ensuring the Quality of Urinary Iodine Procedures

Accurate and precise UI measurement is important to accurately assess the status of iodine nutrition in the US and around the world. Erroneous laboratory data can lead to suboptimal—and potentially harmful—public health interventions. For example, a low estimate of the population median iodine level might prompt inappropriate salt iodization that could result in over-supplementation, a problem that carries its own risks.

CDC established its Ensuring the Quality of Urinary Iodine Procedures (EQUIP) program in 2001 to provide laboratories that measure UI with an independent assessment of their analytical performance, including reference material and technical support to improve laboratory practices. The program, which is voluntary and free-of-charge, currently works with more than 120 iodine laboratories in the U.S. and over 60 countries. Iodine is one of many micronutrients that are the subject of CDC research, population-wide monitoring and laboratory test standardization.
About the Program

The EQUIP program offers three critical services.

First, it provides matrix-matched secondary reference material to laboratories measuring UI. CDC uses inductively coupled plasma-mass spectrometry (ICP-MS) to ensure that UI concentrations are assigned to reference materials with a high degree of accuracy and precision. CDC, in turn, uses the National Institute of Standards and Technology (NIST) reference standard materials (SRM2670a, SRM3668 Level 1 and Level 2) to ensure the accuracy of its own testing.

Second, EQUIP maintains a rigorous performance testing program. Three times a year, CDC sends participating laboratories three to five urine samples that have been spiked with iodine (in a range of 10 to 300 µg/L) for UI analysis. Laboratories are asked to report their test data, along with the limit of detection (LOD) for their analytical method. CDC then returns a comprehensive statistical report allowing each individual laboratory to compare its performance with individual and composite data from all other participating EQUIP laboratories, whose identities are withheld to maintain confidentiality. At the end of each year, laboratories receive a certificate with tabulated progress scores for that year.

Third, EQUIP provides laboratories with analytical guidelines, technical training and consultation upon request. CDC maintains proficiency with iodine spectrophotometric methodologies that are similar to the methods commonly used for UI analysis in laboratories around the world, so its scientists are able to help laboratories eliminate bias and precision problems in their assay systems.

Together, the three components of EQUIP constitute a robust external quality assurance (QA) program intended to increase confidence in UI measurements that are the basis for interventions to eliminate IDD.
Frequently Asked Questions

Q: Is enrolling in EQUIP a long process?
A: No. Once the program receives your application by e-mail or fax, your laboratory will be enrolled immediately.

Q: How much does it cost to participate in the program?
A: Nothing. CDC provides QA materials and technical assistance free-of-charge as part of its effort to help eliminate IDD around the world.

Q: The program description mentions a certificate. Does that mean our laboratory receives certification?
A: No. The certificate is merely a way for laboratories to verify their participation in the program and to track their progress over the course of each year. Participation in this program does not provide or authorize certification or accreditation.

Q: If you calculate progress scores, does this mean our laboratory can fail?
A: No. This is not a pass-fail program. Rather, EQUIP emphasizes measurable and sustained progress.

Q: My laboratory was opened recently and still has many improvements to make. Can we still enroll?
A: Yes. Any laboratory can enroll, and CDC encourages all laboratories performing UI analysis to enroll.

How to Enroll

1. Go to http://www.cdc.gov/labstandards/equip.html and complete the application form.

2. E-mail the completed form to iodinelab@cdc.gov or fax it to (770) 488-4097. A confirmation e-mail will be sent within 72 hours. Your laboratory will be enrolled immediately upon receipt of your form and will receive a set of samples each February, May and August.
Recent Publications


EQUIP has member laboratories in the U.S. and spanning the globe, covering every continent except Antarctica (Figure 1). They include governmental, academic and private-sector laboratories, as well as regional and international laboratories. Many of these laboratories provide training and technical assistance to scientists based in other institutions within the country or in other countries, extending the reach of health promotion activities related to IDD. They also provide their government officials with critical information needed for national fortification efforts.

Collectively, EQUIP members have conducted UI analysis on tens of thousands of specimens to support iodine sufficiency monitoring and public health interventions affecting billions of people throughout the world.

Following are profiles and letters from EQUIP members who provided details of their activities.
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Following are profiles and letters from EQUIP members who provided details of their activities.

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International Council for the Control of Iodine Deficiency Disorders

South Asia Regional Laboratory

We all must feel very proud that we as a team are making a difference in the brain development of millions of children by ensuring adequate iodine intake.

Chandrakant S. Pandav, PhD
Regional Coordinator, ICCIDD
All India Institute of Medical Sciences
New Delhi, India

To assure rigorous laboratory standards for UI analysis and to receive objective verification of test results.

Major Benefits from EQUIP Participation

The ICCIDD South Asia Regional Laboratory, a non-profit, non-governmental laboratory, was established in 1996. Before the initiation of EQUIP, however, the laboratory had no access to an international, external quality assurance program. Participation in EQUIP has enabled the laboratory to authenticate its UI analysis results and, thereby, to gain greater credibility among the investigators who submit urine samples for laboratory testing.

Since joining EQUIP, the laboratory has expanded its upper and lower limits of iodine detection, going from 50 to 300 micrograms/liter to 10 to 400 micrograms/liter.

Iodine Studies and Related Work

The ICCIDD South Asia Regional Laboratory is recognized by the WHO’s Southeast Asia Regional Office as the regional training laboratory for UI and salt iodine estimation. In this capacity, the laboratory has hosted training programs for scientists from countries throughout the region, including Bangladesh, Bhutan, India, Indonesia, Myanmar, Nepal, North Korea, the Republic of Maldives, Sri Lanka and Thailand. Training topics have included iodine sufficiency monitoring, iodine content analysis in salt and QA/QC procedures for universal salt iodization programs, among others.

The laboratory has established iodine testing laboratories in several of these same nations, including Bhutan, Myanmar, Nepal and North Korea.

The laboratory has also provided technical assistance, including UI analysis, to support a number of national and sub-national iodine surveys in the region.

Regional laboratory training, India

Impetus for Joining

To assure rigorous laboratory standards for UI analysis and to receive objective verification of test results.
South African Medical Research Council
Nutritional Intervention Research Unit
Pieter Jooste, PhD
Emmerentia Strydom
Cape Town, South Africa

To take advantage of proficiency testing, exchange information about testing methodologies and standardize test methods.

Major Benefits from EQUIP Participation

The Iodine Nutrition Laboratory migrated from the "Method A" recommended by ICCIDD to manual spectrophotometric analysis with ammonium persulfate digestion and a modified microplate method, adapted to the laboratory’s environment. These technical improvements not only minimize the generation of toxic waste, but also substantially increase test throughput.

The laboratory was able to verify that its urinary iodine measurements obtained using the modified microplate method and the manual spectrophotometric method are on par with measurements obtainable via ICP-MS.

Independent verification of the laboratory’s quality systems and performance has strengthened its position nationally and internationally and has helped to promote SAMRC as a partner in public health research. EQUIP evaluations have helped the Iodine Nutrition Laboratory meet contractual requirements pertaining to quality control for collaborative research projects.

Iodine Studies and Related Work

As a member of the IRLI Network, SAMRC’s Iodine Nutrition Laboratory has completed regional or national iodine sufficiency surveys in 22 countries over the last 12 years. Of these countries, 17 are in Africa and include Angola, Benin, Burkina Faso, Gambia, Ghana, Lesotho, Malawi, Mali, Mozambique, Niger, Rwanda, Algeria, Somalia, Sierra Leone, Sudan, Togo, and Uganda. The laboratory is currently under contract to perform UI analyses for studies and nutritional surveys in Haiti, Nepal, Saudi Arabia and the United Arab Emirates.

In 2005-2006, the laboratory was a key partner in a national food fortification study to determine iodine status and iodized salt coverage in South Africa. In conjunction with this study, the laboratory participated in a number of regional cross-sectional surveys and clinical trials comparing salt iodine content (at production, at the retail level and in households) and nutritional outcomes, including UI measurements and goiter prevalence. This work identified many small pockets of iodine deficiency in South Africa, as well as areas with excessive dietary iodine intake.

The laboratory works with iodine laboratories in Belgium, Indonesia, Kenya and Kuwait on inter-laboratory comparisons of UI analyses.

The laboratory is a founding member of the South African Iodine Deficiency Disorders Network, which coordinates iodine-related public health interventions in South Africa, including surveys, policy formulation and activities to support salt iodization. As part of these activities, for the past five years, the laboratory has been actively training and supporting salt producers in South Africa in the titration method.
The laboratory offers training opportunities and technical assistance to scientists and laboratories worldwide that conduct UI analyses; is a regular collaborator in international iodine research; and regularly participates in the activities of ICCIDD, UNICEF and WHO.

SAMRC works through the Iodine Nutrition Laboratory, Nutritional Intervention Research Unit, to address nutritional imbalances in South Africa, including iodine deficiency. For more information, see www.mrc.ac.za/nutrition.html.

IRLI is a global network of iodine laboratories supporting the monitoring of progress in the global campaign against IDD. The network was established through the joint efforts of CDC, ICCIDD, WHO, The Malnutrition Initiative and UNICEF.

Select References


Van Stuijvenberg ME, Jooste PL, Strydom EE, Faber M, Dhansay MA. Goitre and iodine status of schoolchildren from a previously endemic goitrous area in South Africa: a re-assessment 9 years after the introduction of mandatory salt iodisation. Proceedings of the Micronutrient Forum; 2007 Apr 16-8; Istanbul, Turkey.
University of Papua New Guinea School of Medicine and Health Sciences
Micronutrient Research Laboratory

Our enrollment and performance in EQUIP elevated the status of the Iodine Research Unit, proving beyond a doubt that we are capable of carrying out UI analyses at the highest level of competence.

Victor J. Temple, PhD
Professor of Biochemistry
School of Medicine & Health Sciences
University of Papua New Guinea

Major Benefits from EQUIP Participation

EQUIP provided the urine standards for the laboratory’s internal QC program and also helped to resolve technical problems encountered while setting up the quality control protocols. Having a standard for calibration verification (with a known target value and acceptable range), for example, enabled scientists to identify ambient temperature fluctuations as the cause of variability in UI measurements performed using the “Method A” recommended by ICCIDD. Scientists solved the problem by carrying out UI assays in a water bath maintained at 22-23ºC by the addition of ice cubes.

The laboratory’s participation in EQUIP ensures the high quality of data produced in the Iodine Research Unit and has helped the Unit to secure research grants for the Micronutrient Research Laboratory.

Iodine Studies and Related Work

The Iodine Research Unit has played a key role in providing verifiable scientific data on the iodine nutritional status of vulnerable groups throughout Papua New Guinea, particularly infants, children, and pregnant and lactating women. The unit established a database containing results from monitoring salt iodine content in households and grocery stores, as well as data on the iodine nutritional status of women and children in various regions of the country. The research produced by the unit in the last eight years has shown that the salt iodization program has had some effect, but needs strengthening. Further progress requires continued laboratory-based monitoring.

The Iodine Research Unit conducts periodic workshops on iodine testing in salt for quarantine officers and food inspectors.

The Iodine Research Unit has completed many iodine studies (some of which are referenced here) since participating in the first national micronutrient survey in Papua New Guinea in 2005. Laboratory scientists are currently working on a collaborative project to determine whether successful universal salt iodization in Papua New Guinea guarantees optimal iodine nutrition in mothers and infants.
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Select References


Laboratory scientists, Papua New Guinea

Salt tests positive for iodine
In 2006, the laboratory, together with other five provincial laboratories, revised the national standard method for UI from the chloric acid digestion method to the ammonium persulfate digestion method.

Select References


The laboratory staff’s performance and reliability in testing UI have been significantly improved and enhanced, not only with regards to technical skill, but also in knowledge of laboratory QA methods.

Iodine Studies and Related Work

Reliable UI measurements have been used in assessing the iodine status and thyroid function in pregnant and lactating Chinese women from 2004 to 2009. Results indicated that median UI concentrations in these groups were well below those of the schoolchildren from the same community, the difference between medians, at overall level, being about 50μg/L for the pregnant women and 40μg/L for the lactating women, respectively. Researchers were able to conclude that universal salt iodization in China has brought iodine sufficiency to most of China, but pregnant women in some areas may still risk deficiency and need further supplements. More attention should be given to pregnant and lactating women, and these two groups of the population should be included in a national monitoring program. The laboratory provided their recommendations to the Ministry of Health.

The Challenge of Iodine Deficiency Disorder

EQUIP 10 year anniversary

To improve laboratory analyses quality and to adequately evaluate the iodine status of the Tianjin population.
Frits van der Haar, PhD
Rollins School of Public Health of Emory University, and
Global Public Nutrition Services, LLC
Atlanta, Georgia, USA

I am delighted to congratulate the Inorganic and Radiation Analytical Toxicology Branch at the Division of Laboratory Sciences, CDC with the 10th anniversary of EQUIP. The need for quality data in research has long been known but the introduction of QA services in public nutrition programs is more recent. EQUIP has succeeded in establishing a committed service for supporting laboratory managers around the world in maintaining a high proficiency of UI analyses. Because UI assays are critical for the assessment of iodine nutrition, reliable UI concentrations are of proven value for decisions on the design details of salt iodization strategies aimed at providing optimum dietary iodine in takes.

I have been fortunate to witness a number of instances where EQUIP provided a distinct encouragement to the laboratory staff who were working mostly outside the glamour of the more attention-grabbing disease prevention programs. Memories come to mind, particularly of current iodine laboratories in Macedonia, Romania, Serbia, Kosovo, Kazakhstan, Kyrgyz Republic, Russia and Ukraine. Despite a decade of painful economic transition and working under frequent political upheaval, these laboratories attest to EQUIP’s proven track record of providing reliable information that serves national leaders in making decisions for the welfare and development of each new generation.
With the different salt iodization strategies in the world, the need is increasing for more analyses and insights into the relationships among indicators of iodine supply and status. This area is currently being reviewed by an Iodine Task Force under the guidance of the global Iodine Network, with CDC among the founding members. This technical work depends in no small measure on the steadily grown availability of large population datasets that can be relied upon thanks to the QA from EQUIP. A recent example is the participating laboratory in Tanzania, where salt iodine and UI assays of school children and pregnant women have become incorporated for the first time ever in the thousands of households enrolled in a national Demographic Health Survey.

In closing, once again my heartfelt congratulations to the team who has managed EQUIP so meticulously for ten years! Your service is extraordinary and has outstanding merit. I hope that your program will continue to add its value and be a cornerstone in the building of a world free of IDD.

Arnold Timmer, PhD
Juliawati Untoro, PhD
The United Nations Children’s Fund

Iodine deficiency is the single greatest cause of preventable mental retardation. But the problem is easily and inexpensively prevented by iodizing all salt for human and animal consumption.

By the mid-1990s, many governments made salt iodization an integral part of their national health and nutrition program monitoring. The importance of UI analysis as a guiding indicator for salt iodization and IDD elimination programs therefore relies heavily on the quality of the collection and laboratory analysis. Although often thought to be relatively easy, the collection methods, quality of UI analysis and interpretation of UI data seldom meet appropriate standards.

Progress in monitoring global elimination of iodine deficiency can be noted by the increasing number of countries with data on UI status as well as the establishment of the national and regional laboratory which can accurately perform UI analysis for assessing the iodine status of a population. EQUIP has been contributing significantly to this achievement.

**ICCIDD** is an international organization that promotes optimal iodine nutrition and the elimination of IDD, primarily through universal consumption of iodized salt. ICCIDD’s network consists of over 600 specialists from more than 100 countries. For more information, see www.iccidd.org.

Since the 1950s, UNICEF has provided essential support to global iodine deficiency elimination efforts. Among its numerous efforts, UNICEF also directly assists countries in implementing programs to eliminate iodine deficiency, including the purchase of salt iodization equipment and potassium iodate. For more information, see www.unicef.org/nutrition/index_iodine.html.
UNICEF recognizes and appreciates the great added value that EQUIP brings to the IDD elimination effort. Support from EQUIP has improved the quality performance of UI labs in Central and Eastern Europe and the Commonwealth of Independent States. In collaboration with CDC, a regional resource UI laboratory in Kazakhstan is being upgraded and its capacity strengthened to serve as a resource laboratory for external QC and UI analysis for countries in the region, improving the quality of UI data for this region.

UNICEF greatly values this effort and sincerely hopes and recommends that this service be sustained by CDC as salt iodization efforts are being implemented in more than 120 countries around the world. Ensuring adequate iodine intake is a key factor in the brain development of children and their later school performance. In maturing programs, where a majority of households receive iodized salt, the importance of monitoring UI data for fine-tuning and targeting the salt iodization programs is only increasing. A gradual shift is observed, with more emphasis on monitoring iodine status in other population groups like pregnant women and young children. Obtaining and measuring iodine status among other population groups necessitates good UI laboratory facilities. Continued support from CDC EQUIP in this global effort is required.

Ludmila Ivanova, MD, PhD
The United Nations Children’s Fund

I was first introduced to QA/QC procedures during a three-month laboratory training with the Program Against Micronutrient Malnutrition and CDC in 1993. During this exciting training, our multinational team was taught not only how to perform biological analyses and to produce results but also how to deliver reliable data for policy development and decision making. I realized that one of the keys of sustainable analytical performance is the QA/QC management of the laboratory for iodine analyses.

I returned back to Bulgaria some time later to create the first Bulgarian laboratory for monitoring of IDD elimination under the Ministry of Health. During the same year, universal salt iodization was adopted and we successfully introduced the method for ioduria and analyzed the first urinary samples for national monitoring. We got results but I could not be sure if they were reliable because we were the only ones in the country performing these tests, and I did not have any chance to compare and discuss my performance. And even though we were building experience in iodine analyses, we needed external control and confirmation for our performance. I was looking for opportunities for external QC for ioduria, though without any success until 2000.

It was during my participation at the Salt 2000 Symposium that I learned that CDC, supported by UNICEF, ICCIDD, WHO and others, planned to create a network of international laboratories for iodine, which became known as IRLI. It was so important and came at the right time. The Bulgarian laboratory became a member and resource laboratory of IRLI that same year.

EQUIP was later established in 2001 and we received the first set of QC samples. During the last ten years, EQUIP has ensured the quality performance of UI analyses of many laboratories worldwide and the program...
became an important resource for independent assessment, improving the analytical performance of each laboratory involved.

EQUIP gave me the confidence as an UNICEF consultant to establish iodine monitoring laboratories in Turkmenistan, Azerbaijan and to assist with QC training in laboratories in Romania and Tajikistan. I consider my contact with EQUIP as the most exciting and rewarding experience in my career and strongly believe that the program is an important part in the elimination of IDD worldwide.

Kostas Markou, MD
Irene Mamali, PhD
Division of Endocrinology
University of Patras Medical School
Greece

Our laboratory only recently enrolled in the EQUIP program. Nevertheless, our participation has already proved beneficial for the Greek community. Our laboratory is the only laboratory in Greece which measures UI and studies IDD not just on the national level but also in other countries, such as Azerbaijan, Uzbekistan and Georgia. Thus, it was crucial for us to participate in an external QC program, in addition to the internal QC that was already in place. Thanks to CDC’s EQUIP program our laboratory is now recognized for providing reliable measurements of UI and contributing in the sustainability of iodine sufficiency and the maintenance of public health.
Introduction

UI is the most useful biochemical marker for assessing dietary iodine intake and controlling IDD. This is because the bioavailability of iodine in urine is high; about 90% of iodine consumed is excreted in the urine; and urine samples are easily obtainable without discomfort to the subjects. Additionally, urine samples are stable under the correct storage conditions (ambient temperature and under refrigeration); no preservation or pre-treatment of samples is required; and collected samples (or smaller aliquots) can easily be transported to an analytical laboratory. Furthermore, straightforward analytical techniques are available with acceptable performance and accuracy, and at a relatively low cost.

The three most popular methods for urinary iodine measurement are ammonium persulfate digestion with spectrophotometric detection of the Sandell-Kolthoff (SK) reaction; modified microplate method for the determination of urinary iodine concentration; and ICP-MS. The first method is based on the spectrophotometric determination of the SK reaction. With the introduction of ammonium persulphate as the oxidizing agent to replace hazardous chloric acid during the digestion step, most laboratories opt to use the safer persulfate digestion method. The colorimetric measurement of the SK reaction with microplate applications, either with mild chloric acid or ammonium persulphate, has demonstrated good performance characteristics, and has resulted in the production of comparatively less toxic waste from arsenic trioxide (0.4 mL per test versus 5.0 mL per test).

The second alternative, the microplate method, can be slightly modified to suit laboratory infrastructure by using a heating block instead of the cassette. Advantages of this modification include an even distribution of heat in the heating block; a more representative urine sample (250 μL) used for digestion; a high volume of samples analyzed; and the opportunity to repeat the SK reaction, if necessary, on the same day. Additionally, the modified microplate method showed good agreement with the Technicon autoanalyser method. A comparison between the microplate method, the conventional chloric acid digestion method, and the ICP-MS method yields good correlation coefficients.
The Challenge of Iodine Deficiency Disorder

EQUIP 10 year anniversary

Chemists prepare urinary iodine material for quality assurance.
References


Quality Control Sample Preparation

Reasons For Quality Control

QC gives confidence and validity to results; monitors the performance and output (especially drift) of a process; indicates when problems/deficiencies exist; helps in decision making about results (i.e., acceptable criteria); and provides the statistical basis from which one can judge the results.

Quality Control Materials

When selecting and using QC materials, note the following:

- Composition should be same as patient samples.
- The laboratory procedure manual or manufacturer's manual should specify appropriate QC material for analysis.
- QC materials should be handled as patient samples.
- Mean and standard deviation of QC material must be established before use.
- Control materials with different concentrations must have different lot numbers.

Preparing Bench Quality Control Materials

Step One

Each laboratory can easily prepare urine bench QC material by collecting urine anonymously from several donors. We strongly recommend preparing sufficient volumes of bench QC materials for 2 to 3 years at a time to provide long-term QA for your laboratory and to make the operation as efficient as possible.

Initially, collect urine in 1-L, acid-rinsed polypropylene (PP) or polymethylpentene (PMP) wide-mouth bottles.
The Challenge of Iodine Deficiency Disorder

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Step Two

Prescreen the collected urines to determine the iodine concentrations of each individual sample and to determine how to pool the urines to obtain one low (40-70 ug/L), medium (90-100 ug/L) and high concentration (greater than 100 ug/L). Store urines at 4°C between collection and pooling. Mix the appropriate urines together into a 2-L PP bottle. Ensure adequate mixing of the pool by stirring it on medium speed using a magnetic stirrer bar for 30 minutes. For each pool, aliquot 1.8 mL of urine into a prelabeled 2-mL cryovial and cap the vial. The vials should be frozen at least at -20°C. They can be kept at -20°C for several years.

Step Three

Bench QC materials may be sent to other labs for cross-checking. When performing an interlaboratory exchange of the QC material, we recommend that the samples always be sent on Mondays. This will minimize the chance that the samples will arrive over the weekend. The samples also must be clearly labeled as noninfectious, non-hazardous, human urine/QC material for diagnostic testing. To prevent any delays in the delivery of the samples, the customs requirements for the shipping of the samples into the country should be thoroughly investigated prior to the sample shipment.

Chemists verify samples for urinary iodine analyses.

Chemist screens the collected urine for iodine levels.
Homogeneity Testing of freshly prepared bench QC materials

Pick at random approximately 5% of the total number of vials prepared for each set of materials. Selection is best done by taking a specific number of vials from different locations of each rack that have been prepared with the materials. Analyze those samples in one run to determine whether the QC material is homogeneous. One should not see more than 10% variability from vial to vial (Table 2; Figure 2).

### Table 2. Sample homogeneity testing for iodine

<table>
<thead>
<tr>
<th>Analyte Code</th>
<th>Sample ID</th>
<th>Sequence Number</th>
<th>Final Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine Iodine</td>
<td>Low 1106-001</td>
<td>1</td>
<td>70.10</td>
</tr>
<tr>
<td>Urine Iodine</td>
<td>Low 1106-051</td>
<td>51</td>
<td>70.00</td>
</tr>
<tr>
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<td>Low 1106-101</td>
<td>101</td>
<td>69.90</td>
</tr>
<tr>
<td>Urine Iodine</td>
<td>Low 1106-151</td>
<td>151</td>
<td>69.90</td>
</tr>
<tr>
<td>Urine Iodine</td>
<td>Low 1106-201</td>
<td>201</td>
<td>71.00</td>
</tr>
<tr>
<td>Urine Iodine</td>
<td>Low 1106-251</td>
<td>251</td>
<td>71.00</td>
</tr>
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<td>Urine Iodine</td>
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<td>70.80</td>
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<tr>
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</tr>
<tr>
<td>Urine Iodine</td>
<td>Low 1106-451</td>
<td>451</td>
<td>69.90</td>
</tr>
<tr>
<td>Urine Iodine</td>
<td>Low 1106-500</td>
<td>500</td>
<td>68.30</td>
</tr>
</tbody>
</table>

Mean 70.14  
STD 0.869  
RSD% 1.24
Characterization of bench QC materials

Analyze the newly prepared bench QC materials over 20 days to establish QC limits for each pool. Calculate the mean and standard deviation (SD) for the 20 days, and calculate the mean +/- 2 SD and mean +/- 3 SD to set the limits (Table 3).

Do not analyze patient samples for which results will be reported during this characterization phase. One should have a minimum 10 days’ worth of data to establish preliminary limits before reporting any data for patient samples. When approaching the end of bench QC materials (~40 vials left), prepare new bench QC materials and characterize them while still using the old pools. That way there will be overlapping data from one batch of QC materials to the next.

<table>
<thead>
<tr>
<th>Number of days</th>
<th>Date</th>
<th>Low QC</th>
<th>Mean c</th>
<th>Mean c - 2 SD</th>
<th>Mean c + 2 SD</th>
<th>Mean c - 3 SD</th>
<th>Mean c + 3 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>01/01/11</td>
<td>50.2</td>
<td>50.0</td>
<td>43.1</td>
<td>57.0</td>
<td>39.6</td>
<td>60.5</td>
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<tr>
<td>2</td>
<td>01/02/11</td>
<td>52.3</td>
<td>50.0</td>
<td>43.1</td>
<td>57.0</td>
<td>39.6</td>
<td>60.5</td>
</tr>
<tr>
<td>3</td>
<td>01/03/11</td>
<td>48.3</td>
<td>50.0</td>
<td>43.1</td>
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<td>39.6</td>
<td>60.5</td>
</tr>
<tr>
<td>4</td>
<td>01/04/11</td>
<td>52.0</td>
<td>50.1</td>
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<td>60.5</td>
</tr>
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<td>60.5</td>
</tr>
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<tr>
<td>9</td>
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<tr>
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<tr>
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<td>60.5</td>
</tr>
<tr>
<td>12</td>
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<td>43.1</td>
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<td>60.5</td>
</tr>
<tr>
<td>13</td>
<td>01/13/11</td>
<td>52.0</td>
<td>50.0</td>
<td>43.1</td>
<td>57.0</td>
<td>39.6</td>
<td>60.5</td>
</tr>
<tr>
<td>14</td>
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<td>43.1</td>
<td>57.0</td>
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<td>15</td>
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<td>57.0</td>
<td>39.6</td>
<td>60.5</td>
</tr>
<tr>
<td>16</td>
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<td>50.0</td>
<td>43.1</td>
<td>57.0</td>
<td>39.6</td>
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</tr>
<tr>
<td>17</td>
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<tr>
<td>18</td>
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<td>42.1</td>
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</tr>
<tr>
<td>19</td>
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<td>43.1</td>
<td>57.0</td>
<td>39.6</td>
<td>60.5</td>
</tr>
</tbody>
</table>

Performed: 01/01/11 to 01/20/11

Mean c: 50.0
SD c: 3.5
% CV c: 7.0
Mean c - 2 SD: 43.1
Mean c + 2 SD: 57.0
Mean c - 3 SD: 39.6
Mean c + 3 SD: 60.5
How to Use Bench and Blind Quality Control Materials

Bench Quality Control Materials

Bench QC materials are known to the analyst and inserted at the start of each run (after analyzing the calibration curve and before analyzing any patient samples) and at the end of each run so that judgments can be made on the day of analysis.

Blind Quality Control Materials

Blind QC materials are prepared, tested for homogeneity, and characterized in the same way as bench QC materials. Typically, only two levels of blind QC materials are used: a level representing normal concentrations and a level representing abnormal concentrations (either elevated or depressed). Blind QC materials are labeled so that they are indistinguishable from the patient samples. Because blind QC materials are not known to the analyst, they are processed exactly like patient samples. Only the supervisor reviews the results of the blind QC materials and has the key to decode them. The above procedure for the testing of blind QC materials is difficult when only one person works in the laboratory. One alternative solution would be to have the supervisor pull five random samples to be re-run by the analyst.

Together, bench and blind QC systems help to assess all levels of the analyte concentrations by taking these samples through the complete analytical process. The data from these materials can then be used to estimate methodological imprecision and assess the magnitude of any time-associated trends.
The following is a detailed outline of the three most common urinary iodine methods used by EQUIP participants:

- Ammonium Persulfate Digestion with Spectrophotometric Detection of the Sandell-Kolthoff Reaction.
- Modified Microplate Method for the Determination of Urinary Iodine Concentration.
- Inductively Coupled Plasma-Mass Spectrometry.

Ammonium Persulfate Digestion with Spectrophotometric Detection of the Sandell-Kolthoff Reaction

Advantages of the Method

- Safe digestion process
- Simple manual method (sophisticated instrumentation not needed)
- Inexpensive
- Laboratory-made reagents (no need for diagnostic suppliers)
- Good performance characteristics
- Cost effective and sustainable

Safety Precautions

Follow universal precautions:

Disposable gloves should be worn when handling and pipetting urine specimens before the digestion step.

Arsenic trioxide should be handled with extreme care and disposed of properly, as required by local authorities.

Safety glasses should be worn at all times during the assay and when making up reagents to avoid splashing of any reagents into the eyes.

Broken or chipped glassware should be discarded immediately, including test tubes, which can be easily chipped during washing procedures. Broken glassware should be collected into glassware bins, separate from other laboratory waste, and disposed of properly, as required by local authorities.
Sulfuric acid is highly exothermic. One should always add the acid to the water rather than the water to the acid. Burns from sulfuric acid are serious. The standard first aid treatment for acid spills on the skin is, as for any other corrosive agents, irrigation with large quantities of water. Wear lab coat, gloves and safety glasses when handling and preparing the acid solution.

When work is finished, wipe down all surfaces where urine was handled with 10% (v/v) sodium-hypochlorite solution.

Labeling the reagents with the arrival date and date used is recommended. Keeping an inventory/log sheet for the reagents is also helpful.

**Instrumentation**

Analytical balance (capacity of approximately 250 g and a readability of 0.0001 g). Product#: IL-250U. The Lab Depot, Inc. [www.labdepotinc.com](http://www.labdepotinc.com) or a supplier of your own preference.

Dry heating block. The BenchMark series of Digital DryBath (96X15 mL conical and shape size tube Product#: BSH1004. The Lab Depot, Inc. [www.labdepotinc.com](http://www.labdepotinc.com) or a supplier of your own preference.

Vortex mixers. Product#: M63215, The Lab Depot, Inc. [www.labdepotinc.com](http://www.labdepotinc.com) or a supplier of your own preference.

Stirring hotplates. Product#: sp 133830-33q 10.5x10.5, ceramic. The Lab Depot, Inc. [www.labdepotinc.com](http://www.labdepotinc.com) or a supplier of your own preference.
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Dispensette® III bottletop dispensers. Standard valve, 0.2-2 mL. Product#: 4701320. The Lab Depot, Inc. www.labdepotinc.com or a supplier of your own preference.

Dispensette® III bottletop dispensers. Standard valve, 0.5-5 mL. Product#: 4701330. The Lab Depot, Inc. www.labdepotinc.com or a supplier of your own preference.

Water purification system (NANOpure Diamond Ultrapure Water System, Barnstead International, Bedford, MA or a supplier of your own preference) for providing ultrapure water with a resistivity ≥ 18 MΩ·cm.

Chemicals and Standards

Water, high purity (≥18 MΩ·cm resistivity)

Ammonium peroxydisulfate (NH₄)₂S₂O₈. GFS Chemicals Inc. Columbus, OH USA www.gfschemicals.com or a supplier of your own preference.

Arsenic trioxide (As₂O₃). GFS Chemicals Inc. Columbus, OH USA www.gfschemicals.com or a supplier of your own preference.

Sodium chloride (NaCl). GFS Chemicals Inc. Columbus, OH USA www.gfschemicals.com or a supplier of your own preference.

Concentrated sulfuric acid (H₂SO₄). GFS Chemicals Inc. Columbus, OH USA www.gfschemicals.com or a supplier of your own preference.

Ceric ammonium sulfate dihydrate (NH₄)₄Ce(SO₄)₂·2H₂O. GFS Chemicals Inc. Columbus, OH USA www.gfschemicals.com or a supplier of your own preference.

Stock solution of iodine: CRM - ion chromatography 1000 mg/L iodide in H₂O (Inorganic Ventures - USA, Lakewood, NJ USA www.ivstandards.com). Catalog Number: ICI1-1 and ICI1-5, or a supplier of your own preference.
Potassium iodate (KIO₃). GFS Chemicals Inc. Columbus, OH USA www.gfschemicals.com or a supplier of your own preference.

Reagent Preparation

1. Ammonium persulfate. Dissolve 228.2 g of ammonium peroxydisulfate ((NH₄)₂S₂O₈) in 1 L of 18 MΩ-cm resistivity water. Store in darkness. Keep refrigerated to prevent decomposition (4-10°C). Preferably prepare required volume as need and used reagent within 4 weeks.

2. Arsenious acid. Place 5 g arsenic trioxide (As₂O₃) and 25 g sodium chloride (NaCl) in a clean 1 L volumetric flask, and then add 200 mL 5N sulfuric acid (H₂SO₄). Add about 300 mL of 18 MΩ-cm resistivity water, heat gently, stir to dissolve, and then cool to room temperature. Dilute with 18 MΩ-cm resistivity water to 1 L. Store in darkness. Solution will remain stable for 6 months.

3. Ceric ammonium sulfate solution. Dissolve 24 g ceric ammonium sulfate dihydrate (NH₄Ce(SO₄)₂H₂O) in 1 L of 3.5N sulfuric acid. Make up at least 24 hours before use. Store at room temperature in darkness and solution will remain stable for 6 months.

4. 5N Sulfuric acid (1L). For safety reasons, prepare in an ice-water bath. Slowly add 139 mL concentrated sulfuric acid into a 1-L volumetric polypropylene flask containing about 500 mL of 18 MΩ-cm resistivity water. Dilute to 1 L with 18 MΩ-cm resistivity water. Store in a PP bottle at room temperature and prepare as needed.

5. 3.5N Sulfuric acid (1L). For safety reasons, prepare in an ice-water bath. Slowly add 97 mL concentrated sulfuric acid into a 1-L volumetric VPP flask containing about 500 mL of 18 MΩ-cm resistivity water. Dilute to 1 L with 18 MΩ-cm resistivity water. Store in a PP bottle at room temperature and prepare as needed.
Standards Preparation

Stock Solution of Iodine

CRM - ion chromatography 1000 mg/L Iodide in H₂O (Inorganic Ventures - USA, Lakewood, NJ USA www.ivstandards.com). Catalog Number: ICI1-1 and ICI1-5, or equivalent vendor.

Note: Refer to method on page 45 for the preparation of standards using potassium iodate (KIO₃).

Intermediate Standards Preparation

Add 9 mL of 18 MΩ·cm resistivity water to an acid-rinsed 50 mL tube and then add 1 mL of 1000 mg/L stock standard. Mix well. Store in acid-washed, labeled 50 mL PP tube at room temperature and prepare a fresh set of standards every 6 months.

Working Standards Preparation

Double rinse each flask or tube vigorously with 5% v/v nitric acid followed by a double rinse with 18 MΩ·cm resistivity water. Fill each with 5% v/v nitric acid and allow them to soak for several hours or overnight. Follow with rigorous rinsing of each with 18 MΩ·cm resistivity water.

Fill six labeled, acid-washed 100 mL volumetric flasks with approximately 90 mL of 18 MΩ·cm resistivity water each. Spike each flask with the appropriate volume of the Intermediate Stock Standards Solutions (see Table 4).

<table>
<thead>
<tr>
<th>Working Standards Name</th>
<th>Intermediate Standards (mg/L)</th>
<th>18 MΩ·cm Resistivity Water</th>
<th>Working Standards Concentration (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>STD 1</td>
<td>50 µL</td>
<td>Make up to 100 mL mark</td>
<td>50</td>
</tr>
<tr>
<td>STD 2</td>
<td>100 µL</td>
<td>Make up to 100 mL mark</td>
<td>100</td>
</tr>
<tr>
<td>STD 3</td>
<td>150 µL</td>
<td>Make up to 100 mL mark</td>
<td>150</td>
</tr>
<tr>
<td>STD 4</td>
<td>200 µL</td>
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<tr>
<td>STD 5</td>
<td>250 µL</td>
<td>Make up to 100 mL mark</td>
<td>250</td>
</tr>
<tr>
<td>STD 6</td>
<td>300 µL</td>
<td>Make up to 100 mL mark</td>
<td>300</td>
</tr>
</tbody>
</table>
Mix well. Dilute to 100 mL with 18 MΩ·cm resistivity water. Store in acid-washed, labeled, 50 mL PP tube at room temperature and prepare a fresh set of standards every 6 months.

Pour out small amounts of each calibrator into separate, clean 15 mL PP tubes for daily use in preparing working standards.

Procedures

Allow urine and QC specimens to reach ambient temperature. Vortex the sample well so that no particulates remain on the bottom of the tube before taking an aliquot for analysis.

Pipette 250 µL of each urine sample, working standards and bench QC into a 13 x 100-mm test tube. Pipette all samples in duplicate.

Add 1 mL of ammonium persulfate solution to each tube.

Mix and heat all tubes on a heating block for 60 minutes at 91°- 95°C (digestion step).

After digestion, cool tubes to room temperature.

Add 3.5 mL of arsenious acid solution. Mix and let stand for 15 minutes.

Add 400 µL of ceric ammonium sulfate solution to each tube and quickly mix by vortex or other means (A timer should be used to keep a constant interval of 30 seconds between additions to successive tubes.)

Exactly 30 minutes after the addition of ceric ammonium sulfate to the first tube, read its absorbance at 420 nm in a spectrophotometer. Read successive tubes at the same time intervals as when adding the ceric ammonium sulfate.
Calculation of Results

Graphing software, such as Excel, may be used to construct a standard curve. To create the scatter-plot graph, the log of the absorbance (Abs) at 420 nm is plotted on the y-axis against the standard iodine concentration in µg/L on the x-axis. The equation obtained from the linear trend line of the graph may be used to calculate iodine concentrations of each specimen. Because this is an inverse endpoint reaction, a 1:3 or 1:5 dilution should be performed on the specimens with absorbance values lower than the acceptable standard curve or that calculate concentration >300 µg/L. Ordinary absorbance values range between 0.300 and 1.800 for standards with concentrations between 300 µg/L and 0 µg/L.

Contamination in the Urinary Iodine Laboratory

There are many possible ways of getting contamination in the iodine laboratory. The most common are: equipment, glassware, reagents and water.

It is possible to test suspected contamination problems without having to run a complete digestion procedure. To do this, set up a few tubes with 250 µL 18 MΩ·cm resistivity water and 1 mL ammonium persulfate solution as you would set up a blank sample or 0 µg/L standards in an assay. Add 3.5 mL arsenious acid and let it stand for 15 minutes, then add 400 µL of ceric ammonium sulfate solution to each tube and take note of the time it takes for the color to disappear. If gross contamination is present, the yellow color will fade very quickly.
Modified Microplate Method for the Determination of Urinary Iodine Concentration

Contributed by: Emmerentia Strydom, Pieter Jooste, Medical Research Council, Cape Town, South Africa.

Advantages of the Method

• Simple manual method and safe digestion process
• Laboratory-made reagents (trouble-free preparation of reagents and no need for diagnostic suppliers)
• Ammonium persulfate is a more stable chemical and easy obtainable compared to perchloric acid and potassium chlorate
• Good performance characteristics
• Acceptable inter-laboratory comparison according to the EQUIP proficiency programme
• Adaptation of sample size: 250 µl sample could be reduced to a smaller sample size. For example, a 100 µl can be used as long as a 1:5 ratio with 1.0M ammonium persulfate is applied (or 1:3 ratio with 1.31M ammonium persulfate). This allows the option to repeat the colorimetrical microplate measurement if necessary using the original digested sample on the same day
• Higher volume output. Thus one analyst could easily analyze 300 test samples in one day if all 300 results are within the range of the standard curve
• Cost effective and sustainable
• Easily be upgraded for automation
Method Outline and Principle

The method is outlined in two parts, namely the manual digestion with ammonium persulfate followed by the calorimetrically determination of the S-K reaction using 96 multiwell plates and an absorbance microplate reader at 405 nm.

Manual Digestion of Standards, Controls and Urine Samples

The present digestion step includes the use of 1 mol/L ammonium persulfate as an alternative oxidizing agent to the previous extremely hazardous and explosive chloric acid. In this method the standards, QC controls, and samples are first digested manually with a 1 mol/L ammonium persulfate solution (in a 1:5 ratio) by heating the mixture in a heating block at approximately 91-95°C for 60 minutes to remove possible interfering substances prior to the S–K reaction. Alternatively, urine could be digested with a 1.31 mol/L ammonium persulfate in 1:3 ratio.

Calorimetric Determination of the Sandell-Kolthoff Reaction with a Microplate Reader

Iodide is the catalyst in the reduction of ceric ammonium sulfate (yellow) to the cerous form (colorless), and is detected by the rate of color disappearance in the S–K reaction. In the adaptation of the S–K reaction as described in the ammonium persulphate digestion microplate (APDM) method, small volumes of the digested urine samples and reagents are pipetted into the wells of a 96-multiwell polystyrene microplate. The microplate is then incubated for 30 minutes at a constant temperature (30 minutes at room temperature or 25 minute at 30°C) and the colorimetric reaction takes place in the wells of the microplate. After the incubation period, the inverse endpoint assay is measured colorimetrically with an absorbance microplate reader at 405 nm. The intensity of the color is inversely proportional to the iodine concentration and the iodine concentration is normally expressed in µg/L.

Scope

This method is applicable for detecting iodine in random urine, 24 hour collections of urine, as well as water samples (drinking water, water for agricultural purposes and surface seawater). Although not highly recommended, this method could be adapted for determining the iodine content in iodized salt samples by performing various dilutions (approximately 200-fold to 500-fold) of the salt sample. In this method the detailed measurement for UI is described, which is also applicable to iodine analysis for water samples. Because the iodine concentration of water samples is normally low, it is recommended to include standards with lower iodine concentrations ranging from 0 to 160 µg/L in the standard curve. This adaptation of the standard curve will yield better linearity and more accuracy at the lower end of the fitted curve.

The limit of detection (LOD) ranges from approximately 5 to 18 µg/L for the microplate method. Calibration (standard) curves have demonstrated acceptable correlation coefficients of linearity for iodine calibrators (standards) ranging from 0 to 300 µg/L, repeatedly. Because of this, 2-fold and 5-fold dilutions with water should be performed on the urine specimens with absorbance values lower than the acceptable calibration curve or on those samples calculated to have an iodine concentration above the highest concentration of the calibration curve (e.g. >300 µg/L.) After diluted sample are assayed, the iodine concentration needs to be calculated using the appropriate dilution factor (e.g. 2 or 5) to determine the original and absolute iodine concentration of the urine specimen.
Safety Precautions

Follow universal precautions:

Disposable gloves should be worn when handling and pipetting urine specimens before the digestion step.

Arsenic trioxide should be handled with extreme care and disposed of properly, as required by local authorities.

Safety glasses should be worn at all times during the assay and when making up reagents to avoid splashing reagents into the eyes.

Sulfuric acid is highly exothermic. One should always add the acid to the water rather than the water to the acid. Burns from sulfuric acid are serious. The standard first aid treatment for acid spills on the skin is, as for other corrosive agents, irrigation with large quantities of water. Wear lab coat, gloves, and safety glasses when handling and preparing the acid solution.

Broken or chipped glassware should be discarded immediately, including test tubes, which can be easily chipped during washing procedures. Broken glassware should be collected into glassware bins, separate from other laboratory waste, and disposed of properly, as required by local authorities.

When work is finished, wipe down all surfaces where urine was handled with 10% (v/v) sodium-hypochlorite solution.

Labeling the reagents with the arrival date and date used is recommended. Keeping an inventory/log sheet for the reagents is also helpful.

Instrumentation

Analytical balance (capacity of approximately 250 g and a readability of 0.0001 g). Product#: IL-250U. The Lab Depot, Inc. www.labdepotinc.com or a supplier of your own preference.

Absorbance microplate reader (e.g. Biotek Elx808) with a 405 nm filter and with basic data analysis and curve fitting software (e.g. KC junior) which is connected to a computer. BioTek www.biotek.com or a supplier of your own preference.

Water purification system (NANOpure Diamond Ultrapure Water System, Barnstead International, Bedford, MA or a supplier of your own preference) for providing ultrapure water with a resistivity ≥ 18 MΩ·cm.
Microtiter plate shaker, which can accommodate 4 or at least 2 microplates. A digital high performance temperature controlled shaker is optional and only necessary in laboratories which experience fluctuation in temperatures constantly through the day or from day to day. IKA www.ika.net or a supplier of your own preference.

Dry heating block with preferably 210 holes or at least 96 holes. Diameter of holes should be 16 mm or 13 mm as long as it could accommodate the corresponding durable culture tubes. Alternatively, a water bath with the appropriate stainless racks could be used for the digestion procedure. Product#: BSH1004. The Lab Depot, Inc. www.labdepotinc.com or a supplier of your own preference.


Stirring hotplates. Product#: sp 133830-33q 10.5x10.5, ceramic. The Lab Depot, Inc. www.labdepotinc.com or a supplier of your own preference.

A thermometer for the heating block. Product#: HI98501. The Lab Depot, Inc. www.labdepotinc.com or a supplier of your own preference.


The use of a fume hood or a good ventilation system is recommended for the preparation of certain reagents as well as for the digestion process.

Dispensette® III bottletop dispensers. Standard valve, 0.2-2mL. Product#: 4701320. The Lab Depot, Inc. www.labdepotinc.com or a supplier of your own preference.

Dispensette® III bottletop dispensers. Standard valve, 0.5-5mL. Product#: 4701330. The Lab Depot, Inc. www.labdepotinc.com or a supplier of your own preference.

Eppendorf adjustable-volume pipette. www.eppendorf.com or a supplier of your own preference.

Eppendorf multichannel pipette (8 channel). www.eppendorf.com or a supplier of your own preference.

Pipette tips. www.eppendorf.com or a supplier of your own preference.

Reagent reservoirs with tight lids for the channel pipettes (Texan TM reservoir for multichannel pipettes). Catalog#: R1525 or R1400, www.sigma-aldrich.com or a supplier of your own preference.

96 Multiwell plate stands. Catalog#: Z363359, www.sigma-aldrich.com or a supplier of your own preference.
Chemicals and Standards

Water, high purity (≥ 18 MΩ·cm resistivity).

Ammonium peroxydisulfate (NH$_4$)$_2$S$_2$O$_8$. GFS Chemicals Inc. Columbus, OH USA [www.gfschemicals.com](http://www.gfschemicals.com) or a supplier of your own preference.

Arsenic trioxide (As$_2$O$_3$). GFS Chemicals Inc. Columbus, OH USA [www.gfschemicals.com](http://www.gfschemicals.com) or a supplier of your own preference.

Sodium chloride (NaCl). GFS Chemicals Inc. Columbus, OH USA [www.gfschemicals.com](http://www.gfschemicals.com) or a supplier of your own preference.

Sodium hydroxide (NaOH). GFS Chemicals Inc. Columbus, OH USA [www.gfschemicals.com](http://www.gfschemicals.com) or a supplier of your own preference.

Concentrated sulfuric acid (H$_2$SO$_4$). GFS Chemicals Inc. Columbus, OH USA [www.gfschemicals.com](http://www.gfschemicals.com) or a supplier of your own preference.

Ceric ammonium sulfate dihydrate (NH$_4$)$_4$Ce(SO$_4$)$_4$.2H$_2$O. GFS Chemicals Inc. Columbus, OH USA [www.gfschemicals.com](http://www.gfschemicals.com) or a supplier of your own preference.

Stock solution of iodine: CRM - ion chromatography 1000 mg/L iodide in H$_2$O (Inorganic Ventures - USA, Lakewood, NJ USA [www.ivstandards.com](http://www.ivstandards.com)). Catalog Number: ICI1-1 and ICI1-5, or a supplier of your own preference.

Potassium iodate (KIO$_3$). GFS Chemicals Inc. Columbus, OH USA [www.gfschemicals.com](http://www.gfschemicals.com) or a supplier of your own preference.

1M (1N) nitric acid (HNO$_3$). GFS Chemicals Inc. Columbus, OH USA [www.gfschemicals.com](http://www.gfschemicals.com) or a supplier of your own preference.

Sodium carbonate (soda ash)(Na$_2$CO$_3$). GFS Chemicals Inc. Columbus, OH USA [www.gfschemicals.com](http://www.gfschemicals.com) or a supplier of your own preference.

All chemicals used for the preparation of reagents and standards should be analytical grade and obtained from reputable suppliers. Use high purity (≥ 18 MΩ·cm resistivity water) to prepare reagents, standards, and for the dilutions of controls and samples, as well as for the preparation of 1M (molar) nitric acid. Additionally, all chemicals should be stored in a cool dark place at room temperature except for the ammonium persulfate which should be store in a refrigerator (5°C) to prevent decomposition. Ammonium persulfate decomposes when exposed to moisture and heat. If possible, store potassium iodate in a desiccator at room temperature (18-24°C) or in a refrigerator. Labeling of chemicals is important to keep track of possible decomposition, contamination, and expiration. Thus label chemicals as follows: arrival date, opening date (first used), and expected expiration date.
Reagent Preparation

1. Ammonium persulfate (1 mol/L). Dissolve 228.2 g of ammonium peroxydisulfate (\(\text{(NH}_4)_5\text{S}_2\text{O}_8\)) in 1 L of ≥ 18 MΩ·cm resistivity water. Store in the dark. Keep refrigerated to prevent decomposition (4-10°C). Preferably prepare required volume as need and use reagent within 4 weeks.

2. Arsenious acid (0.05 mol/L). Dissolve 5 g arsenic trioxide in 100 mL of 0.875 mol/L sodium hydroxide solution. Add 16 mL of concentrated sulfuric acid slowly to the solution in an ice bath (a bucket with crushed ice or cold water with ice blocks will be sufficient), while stirring solution constantly on a magnetic stirrer. After cooling, remove the ice bath and add 12.5 g of sodium chloride to the solution and dilute the solution to the 500 mL mark with 18 MΩ·cm resistivity water. Filter the solution with qualitative or quantitative filter paper circles with a medium to fast flow rate and good particle retention characteristics. Store in the dark away from light at room temperature. Solution will remain stable for 6 months.

3. Ceric ammonium sulfate solution (0.019 mol/L). Dissolve 6 g ceric ammonium sulfate dihydrate in 500 mL of 1.75 mol/L (3.5N) sulfuric acid. Store in a dark bottle away from light at room temperature. Solution will remain stable for 6-12 months. Alternatively, prepare a lower concentration of ceric ammonium sulfate (e.g. 5g/500 ml) if the optical density of the zero standard is constantly too high and > 2.00 after 30 to 35 minutes of incubation. This happens occasionally in laboratories with colder room temperatures (e.g. 16-20°C).

4. Sodium hydroxide (0.875 mol/L). Dissolve 8.75 g of sodium hydroxide pellets in 250 mL ≥ 18 MΩ·cm resistivity water. Preferably prepare solution as needed. Store solution in a cool dark place. Solution is stable for 3 months. Remember that low strength of NaOH is subject to a higher percentage deterioration on exposure to air.

5. Sulfuric acid (3.5N) (1.75 mol/L). For safety reasons, prepare in an ice-water bath. Slowly add 97 mL concentrated sulfuric acid into a 1-L volumetric PP flask containing about 500 mL of 18 MΩ·cm resistivity water. Dilute to 1 L with ≥ 18 MΩ·cm resistivity water. Store in a PP bottle at room temperature and prepare as needed. Stable indefinitely, but it is advisable to prepare fresh sulfuric acid every 12-24 months especially when acid is contaminated with debris. Sometimes the deterioration of the screw caps of the storage bottles is responsible for the fine plastic particles in the acid.

Most of the time, prepare reagents as needed, minimizing decomposition or exposure to unnecessary environmental factors (e.g. light and high ambient temperatures). To prepare smaller or larger volumes of reagent solutions, add proportionally the required mass quantities (g) or volumes (mL) of the solution constituents.
Standards Preparation

Stock Standard Solution

Dissolve 1.68 g KIO₃ in deionized water to a final volume of 1000 mL in a volumetric flask. This solution is equivalent to 1000 µg iodine/mL (1000000 µg/L). Mix well.

Intermediate Standards Preparation

Prepare a 1000-fold dilution of stock standard-A. Thus 1 mL of stock standard-A diluted to 1000 mL in a volumetric flask will be equivalent to a concentration of 1 µg iodine/mL (1000 µg/L). Mix well. Store all stock standards in acid-washed PP bottles, high density poly-ethylene, or low density polyethylene (LDPE) bottles.

Note:

1.68 mg contains 1.0 mg iodine
1,000,000 µg = 1000 mg = 1 g
1 µg/mL (ppm) = 100 µg/dL = 1000 µg/L
1 µg/L = 0.0079 µmol/L ~ multiply µg/L by 1/127, where 127 is the atomic weight of iodine and both units are expressed as per liter. Thus 1 µmol/L = 127 µg/L.
Working Standards Preparation

After deciding the number of standards and the concentrations required for the standard curve, prepare the selected standards as indicated in Table 5. Then pipette the appropriate volume of intermediate standard (1000 µg/L) into acid-washed volumetric flasks (50 ml). Add slowly ≥ 18 MΩ·cm resistivity water to each flask and make up to the 50 ml mark. Working standards 20, 40, 80, 120, 200, and 300 µg/L are recommended for the calibration curve.

<table>
<thead>
<tr>
<th>Working Standards Name</th>
<th>Intermediate Standard (µg/L)</th>
<th>18 MΩ·cm Resistivity Water</th>
<th>Iodine Concentration (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>STD 2</td>
<td>0.1mL</td>
<td>Make up to 50 mL mark</td>
<td>2</td>
</tr>
<tr>
<td>STD 5</td>
<td>0.25mL</td>
<td>Make up to 50 mL mark</td>
<td>5</td>
</tr>
<tr>
<td>STD 10</td>
<td>0.5mL</td>
<td>Make up to 50 mL mark</td>
<td>10</td>
</tr>
<tr>
<td>STD 20</td>
<td>1.0mL</td>
<td>Make up to 50 mL mark</td>
<td>20</td>
</tr>
<tr>
<td>STD 40</td>
<td>2.0mL</td>
<td>Make up to 50 mL mark</td>
<td>40</td>
</tr>
<tr>
<td>STD 80</td>
<td>4.0mL</td>
<td>Make up to 50 mL mark</td>
<td>80</td>
</tr>
<tr>
<td>STD 120</td>
<td>6.0mL</td>
<td>Make up to 50 mL mark</td>
<td>120</td>
</tr>
<tr>
<td>STD 160</td>
<td>8.0mL</td>
<td>Make up to 50 mL mark</td>
<td>160</td>
</tr>
<tr>
<td>STD 200</td>
<td>10.0mL</td>
<td>Make up to 50 mL mark</td>
<td>200</td>
</tr>
<tr>
<td>STD 280</td>
<td>14.0mL</td>
<td>Make up to 50 mL mark</td>
<td>280</td>
</tr>
<tr>
<td>STD 300</td>
<td>15.0mL</td>
<td>Make up to 50 mL mark</td>
<td>300</td>
</tr>
<tr>
<td>STD 400</td>
<td>20.0mL</td>
<td>Make up to 50 mL mark</td>
<td>400</td>
</tr>
</tbody>
</table>
Use stopper flasks and mix well by hand. Alternatively, add a magnetic stir-bar to flask and mix standards by using a multi-position magnetic stirrer. Pour out into dry 50 mL labeled centrifuge cups and store in a refrigerator.

Pour out small amounts of each calibrator into separate clean 15 mL labeled tubes for daily use.

Store all standards away from light in a refrigerator, and prepare a fresh set of standards every 6 months. Standards may be stored at controlled room temperature, but refrigeration is recommended for countries with hot climates.

Use working standards 20, 40, 80, 120, 200, and 300 µg/L for the calibration curve, and use working standards 160 and 400 µg/L as internal controls. Samples with too little urine for retesting will be accepted with values up to 400 µg/L if STD 400 showed acceptable performance; this means that the LOD was not lower than 0.150 and the extrapolated concentration of STD 400 was within 7.5% of the expected value.

Working standard 2, 5, and 10 µg/L are occasionally used to re-evaluate the accuracy and detection limits at the lower end of the standard curve.

Furthermore, these low standards are included in the adaptation of the standard curve for water analysis studies or surveys of severely to moderate iodine deficient populations. The span of these standard curves will range from 0 to 120, 160, or 200 µg/L, which will yield better accuracy and coefficient of variation of the expected concentrations of the unknown samples.

**Procedures**

Allow urine, standards, and QC to reach ambient temperature. Mix urine thoroughly to suspend sediment before taking an aliquot for analysis.

Pipette 250 µL of each urine sample, working standards, including the blank (zero standard) and QC into the appropriate round bottom glass tubes. For the microplate application, the blanks, standards, and QC samples should not be more than 24 samples, leaving 72 test samples per microplate run. Working standards 0, 20, 40, 80, 120, 200, and 300 µg/L are recommended for the calibration curve. In addition, working standards 160 and 400 µg/L are used as internal controls.

Add 1 ml of 1 mol/L ammonium persulfate solution to each tube.

Mix and heat all tubes on a heating block for 60 minutes at 91°- 95°C (digestion step). Alternatively a waterbath can be used.

Cool tubes to room temperature. After this manual digestion step the SK reaction steps follow as described in the APDM method.
After cooling down, 50 µl of the digested samples, standards, and controls should be transferred to the appropriate wells of a polystyrene microplate as indicated in Table 6. For transferring the 50 µl aliquots use a positive displacement pipette with a long capillary tip or research pipette. Use a channel pipette to add the reagents to the wells of the microplate.

<table>
<thead>
<tr>
<th>Microplate layout</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1_A</td>
<td>STD 0</td>
<td>STD 120</td>
<td>SC_160</td>
<td>1</td>
<td>9</td>
<td>17</td>
<td>25</td>
<td>33</td>
<td>41</td>
<td>49</td>
<td>57</td>
<td>65</td>
</tr>
<tr>
<td>2_B</td>
<td>STD 0</td>
<td>STD 120</td>
<td>SC_160</td>
<td>2</td>
<td>10</td>
<td>18</td>
<td>26</td>
<td>34</td>
<td>42</td>
<td>50</td>
<td>58</td>
<td>66</td>
</tr>
<tr>
<td>3_C</td>
<td>STD 20</td>
<td>STD 200</td>
<td>UIC_1</td>
<td>3</td>
<td>11</td>
<td>19</td>
<td>27</td>
<td>35</td>
<td>43</td>
<td>51</td>
<td>59</td>
<td>67</td>
</tr>
<tr>
<td>4_D</td>
<td>STD 20</td>
<td>STD 200</td>
<td>UIC_2</td>
<td>4</td>
<td>12</td>
<td>20</td>
<td>28</td>
<td>36</td>
<td>44</td>
<td>52</td>
<td>60</td>
<td>68</td>
</tr>
<tr>
<td>5_E</td>
<td>STD 40</td>
<td>STD 300</td>
<td>UIC_3</td>
<td>5</td>
<td>13</td>
<td>21</td>
<td>29</td>
<td>37</td>
<td>45</td>
<td>53</td>
<td>61</td>
<td>69</td>
</tr>
<tr>
<td>6_F</td>
<td>STD 40</td>
<td>STD 300</td>
<td>UIC_3</td>
<td>6</td>
<td>14</td>
<td>22</td>
<td>30</td>
<td>38</td>
<td>46</td>
<td>54</td>
<td>62</td>
<td>70</td>
</tr>
<tr>
<td>7_G</td>
<td>STD 80</td>
<td>SC_400</td>
<td>UIC_3</td>
<td>7</td>
<td>15</td>
<td>23</td>
<td>31</td>
<td>39</td>
<td>47</td>
<td>55</td>
<td>63</td>
<td>71</td>
</tr>
<tr>
<td>8_H</td>
<td>STD 80</td>
<td>SC_400</td>
<td>UIC_3</td>
<td>8</td>
<td>16</td>
<td>24</td>
<td>32</td>
<td>40</td>
<td>48</td>
<td>56</td>
<td>64</td>
<td>72</td>
</tr>
</tbody>
</table>

Note:
- Wells 1 to 14 contain the working standards for the standard curve.
- Wells 15 to 18 contain the internal standard controls (SC).
- Wells 19 to 24 contain the control samples (UIC are internal bench controls).
- Wells 25 to 80 contain the test samples (n=72).

Add 100 µl of arsenic acid solution to each well. Cover the microplate with a dedicated polystyrene microplate lid and mix gently by rotating with a back and forth swirling motion for approximately 15-60 seconds. Alternatively use a microtiter plate shaker.

Add 50 µl ceric ammonium sulfate solution quickly (within one minute) to each well. With practice, the ceric ammonium sulfate could easily be added within 40 seconds. Since this is an inverse chemical reaction with some kinetic properties it is important to be consistent in the order of reagent addition to the wells and the rate of pipetting.

Cover the microplate with a dedicated polystyrene microplate lid and mix thoroughly on a microtiter plate shaker (e.g. at a speed of 400-600 rpm), while incubating the microplate for 30 minutes at room temperature (or 25 minutes at 30°C).

Exactly 30 minutes after the incubation, read the absorbance at 405 nm (or 420 nm) with a microplate reader. Remove the lid from the plate when reading the absorbance. Most basic microplate readers with a filter wheel come with a 405 nm filter, but the more advanced microplate readers have a monochromator system where the 420 nm wavelength can be selected.
Calculation of Results

Most microplate readers (e.g., Biotek ELx808) can be connected to computers with basic data analysis and curve fitting software (e.g., KC Junior). This software is setup initially by programming an endpoint assay protocol which will include the layout of the microplate (the position of the standards, the controls, and samples), and the curve fitting properties. With KC Junior, the linear curve fitting is used with the log of the absorbance at 405 nm plotted on the y-axis against the standard iodine concentration (linear) in µg/L on the x-axis. Results are either printed or exported to Excel and merged with the corresponding sample identification name or code.

For microplate readers without the curve fitting software, the optical densities (absorbance readings) of the standards are used to construct a standard curve by using graphing software, such as Excel. To create the scatter-plot graph, the log of the absorbance at 405 nm is plotted on the x-axis against the standard iodine concentration in µg/L on the y-axis. The equation obtained from the linear trend line of the graph may be used to calculate standard iodine concentrations of each specimen. Ordinary absorbance values range between 0.300 and 1.800 for standards with concentrations between 300 µg/L and 0 µg/L.

Performance Characteristics

Determine the performance characteristics of the analytical method annually or as required by a proposed research study or contractual requirements. These performance characteristics include the precision, accuracy, detection limits, and recovery, which includes the linearity of the recovery and the linearity of the standard curve.

Precision

Determine the intra-assay precision (repeatability) for pooled urine samples with low, medium, and high concentrations of iodine by analyzing each sample 8 or 16 times (8 or 16 digested samples) on the same microplate per day and across three consecutive days. Using the same pooled urine samples, analyze in duplicate on the same microplate for the inter-assay over a period of 20 working days (or at least 20 microplate runs over at least two weeks). The coefficient variation for the intra-assays and the inter-assays are calculated for each pooled urine sample.

Accuracy and Quality Assurance

For the internal verification of the accuracy of the analytical method, analyze the certified NIST SRM 2670a (elevated) freeze-dried urine, which has a certified concentration value of 88.2 ± 2.2 µg/L for iodine, in triplicate over three consecutive days (or three consecutive microplate runs). Through participating in the EQUIP proficiency program the laboratory performance and accuracy is externally evaluated and compared to many iodine laboratories globally three times per year.
Recovery

Analyze eight different urine samples (with iodine concentrations ranging from approximately 20 to 200 µg/L) in triplicate which are spiked with a known iodate solution, and a separate set of the same samples which were spiked with a zero standard ≥ 18 MΩ·cm resistivity water. The iodate-added urine is prepared by adding a given volume (1 volume of the 400 µg/L iodate standard and 9 volumes urine) of potassium iodate solution (400 µg/L) to the urine samples. For the water-urine solution, water is added instead of the potassium iodate solution. Compare the results of the two sets of samples.

Linearity of Recovery

Pooled urine samples with low, medium, and high concentrations of iodine are serially diluted and analyzed in triplicate. Results are then multiplied with the dilution factors and compared with the expected (theoretical) value.
Linearity of Standard Curve

Determine the correlation coefficient for the linearity of standard curves by using at least six standards (calibrators). This is applicable for all the adaptations of the standard curve.

Each laboratory has to evaluate the method first to determine the performance characteristics and incubation time, temperature and reagent concentrations that suit the laboratory and equipment for optimal performance of the method. To ensure that iodine-free water is used, especially if iodine contamination is expected, the tap water, purified water, distilled water, and deionized water should be tested for iodine. Durable labware, laboratory consumables (e.g. collection cups, tubes, tips, Pasteur pipettes and microplates), laboratory soaps, hands soaps and disinfectants, used in the iodine laboratories or used at the sample collection sites, should be iodine-free and, if required, tested for possible iodine contaminants.

Inductively Coupled Plasma-Mass Spectrometry
Advantages of the Method

• Measure isotope masses (isotope studies, spectral simplicity)
• High sensitivity
• Low detection limits
• Wide linear range (from ppt to ppm)
• Fast scanning
• High sample throughput
• Minimal sample preparation

Method Outline and Principle

ICP-MS is a multi-element analytical technique. Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio-frequency power with flowing argon, plasma is created in which the predominant species are positive argon ions and electrons. The sample passes through a region of the plasma that has a temperature of 6000-8000 °K. The thermal energy atomizes the sample and then ionizes the atoms. The ions and the argon enter the mass spectrometer through the interface that separates the ICP, which operates at atmospheric pressure, from the MS, which operates at a pressure of 10⁻⁶ torr. The MS permits rapid-sequence ion detection at each mass, which allows determination.
of individual isotopes of an element. Electrical signals from the ion detection are processed into digital infor-
mation that is used to first indicate the intensity of the ions and then the concentration of the element. Urine
samples are diluted 1+9 with 1% (v/v) tetramethylammonium hydroxide (TMAH) containing tellurium for
internal standardization.

Safety Precautions

Follow universal precautions:

Wear gloves, a lab coat, and safety glasses while handling human urine. Place in a biohazard autoclave bag all
disposable plastic, glass, and paper (e.g., pipette tips, autosampler tubes, gloves, etc.) that contact urine. Keep
these bags in appropriate containers until they are sealed and autoclaved. When work is finished, wipe down all
surfaces where urine was handled with 10% (v/v) sodium-hypochlorite solution. Use of the foot pedal on the
Micromedic Digiflex™ reduces the analyst’s contact with work surfaces that have been in contact with urine and
also keeps the analyst’s hands free to hold the specimen cups and autosampler tubes.

Exercise special care when handling and dispensing concentrated nitric acid. Always remember to add the acid
to the water. Nitric acid is a caustic chemical that is capable of severe eye and skin damage. Wear powder-free
gloves, a lab coat and safety glasses.

If nitric acid comes in contact with any part of the body, quickly wash the affected area of the body with copious
quantities of water for at least 15 minutes.

Micromedic Digiflex™ automatic pipette equipped with 10.0-mL dispensing syringe, 2,000 mL sampling syringe, 0.75-mm tip and the foot pedal, if desired. Micromedic Systems, Inc., Horsham, PA or a supplier of your own preference.

Milli-Q® Plus water-purification system. Millipore Corporation, Bedford, MA or a supplier of your own preference.

Eppendorf™ fixed-volume micropipettes: 1,000, 500, 100, 50, and 10 mL volumes. Brinkmann Instruments, Inc., Westbury, NY or a supplier of your own preference.


Water purification system (NANOpure Diamond Ultrapure Water System, Barnstead International, Bedford, MA or a supplier of your own preference) for providing ultrapure water with a resistivity ≥18 MΩ·cm.

Analytical balance for routine weighing of material to the nearest tenth of a gram and with a loading capacity of at least 200 g. The Lab Depot, Inc. www.labdepotinc.com or a supplier of your own preference.

Acid-cleaned PP and PMP volumetric flasks (100-mL, 2,000-mL volumes) for standards and QC preparation. One 10-mL acid-cleaned volumetric flask. Three 2-mL acid-cleaned Teflon™ bottles (one for diluent, one for base urine pool, and one for the 2% Triton X-100™/5% GFS™ double-distilled, concentrated nitric-acid solution). Five 1-L acid-cleaned, wide-mouth PP (or PMP), (four for base urine collection from donors and one for rinse solution). Rinse containers and flasks with reagent-grade, concentrated nitric acid, then rinse rigorously with 18 MΩ·cm Milli-Q® water.

Chemicals and Standards

Water, high purity (≥18 MΩ·cm resistivity).

TritonX-100™ (Aldrich Chemical Co., Milwaukee, WI, or any source whose product is low in trace-metal contamination).

Concentrated (16M or ~70%) nitric acid. GFS Chemicals Inc. Columbus, OH USA www.gfschemicals.com or a supplier of your own preference (If other stock concentrations are used, volumes must be adjusted accordingly).

Ethyl alcohol (ethanol) (C2H5OH), ACS/USP absolute, anhydrous, 200 proof. Aaper-Pharmco Products,
Inc., Shelbyville, KY or a supplier of your own preference.

Stock solution of iodine: CRM - ion chromatography 1000 mg/L iodide in H₂O (Inorganic Ventures - USA, Lakewood, NJ USA www.ivstandards.com), Catalog Number: ICI1-1 and ICI1-5, or a supplier of your own preference.

Tellurium (Te) stock standard: 1,000 mg Te / L in 2-10% HNO₃ (Inorganic Ventures - USA, Lakewood, NJ USA www.ivstandards.com), Catalog Number: XXX, or a supplier of your own preference.

Ethylene diaminetetraacetic Acid (EDTA Sigma-Aldrich Chemicals, St. Louis, MO or a supplier of your own preference.

Tetramethylammonium hydroxide (TMAH), 25% w/w, or equivalent. AlfaAesar, 30 Bond St., WardHill, MA 01835 or a supplier of your own preference.

Sodium hypochlorite (bleach) or equivalent for preparation of 10% bleach solution used for biological decontamination. GFS Chemicals Inc. Columbus, OH USA www.gfschemicals.com or a supplier of your own preference.

Liquid argon (supplied by Specialty Gases or other contract agency) equipped with approved gas regulator. Matheson Gas Products, Secaucus, NJ or supplier of your own preference.

Reagent Preparation

1. Diluent (1% TMAH, 0.01% Triton X-100™ plus 10 µg Te/L). The diluent used in this method is an aqueous solution of 1.0% (v/v) TMAH, 0.01% Triton-X-100™ and 10 µg/L Te. To prepare, acid-rinse a 2-L Teflon™ container and partially fill with ultrapure, 18 MΩ·cm water. Add 80 mL of 25% (v/v) TMAH 10 mL of 2% Triton-X-100™ and 20 µl Te. Dilute to 2L with ultrapure water. Store at room temperature and prepare as needed.

2. ICP-MS rinse solution (1% TMAH plus 0.1% Triton X-100™). The wash solution used in this method is an aqueous solution of 0.1% Triton X-100™ and 1.0% TMAH. Pump this solution into the sample introduction system between samples to prevent carryover of the analytes of interest from one sample measurement to the next. To prepare, acid-rinse a 2-L Teflon™ container and partially fill with ultrapure18 MΩ·cm water. Add 80 mL of 25% (v/v) TMAH and 100 mL of 2% Triton X-100™/5% GFS™ double-distilled, concentrated nitric-acid solution. Dilute to 2L with ultrapure water. Store at room temperature and prepare as needed.

3. Base urine. The base urine used in this method is a pool of urine collected from several donors. Combine this base urine with intermediate working standards during the dilution process just prior to analysis. Initially, collect urine in 1-L, acid-rinsed, wide-mouth bottles. After the urine is collected from donors, mix together into a Teflon™ bottle. Aliquot into 15-mL PP centrifuge tubes; use one tube at a time. Store at 4°C between uses.
Standards Preparation

Stock Solution of Iodine

CRM - ion chromatography 1000 mg/L iodide in H₂O. Inorganic Ventures - USA, Lakewood, NJ USA
www.ivstandards.com. Catalog Number: ICI1-1 and ICI1-5, or equivalent vendor.

Intermediate Stock Standards Preparation

Iodine intermediate stock calibrator and calibration verification solution A ('IOD-A', 100 mg/L iodine in water):

Partially fill an acid-rinsed 100mL glass volumetric flask with ≥18 MΩ-cm resistivity water. Add 10 mL of
1000 mg/L stock standard. Dilute to the 100 mL mark with ≥18 MΩ-cm resistivity water. Mix well before use or
storage. Store an aliquot of this solution in a properly labeled glass bottle at refrigerated temperatures (~2-4ºC)
and prepare a fresh set of standards every 6 months.

Iodine intermediate stock calibrator solution B ('IOD-B', 10 mg/L iodine in water):

Partially fill an acid-rinsed 100 mL glass volumetric flask with ≥18 MΩ-cm water. Mix the iodine Intermediate
Stock Solution A ('IOD-A') well then pipette 10 mL of it into the partially filled, 100 mL flask. Dilute to the 100
mL mark with ≥18 MΩ-cm water. Mix well before use or storage. Store an aliquot of this solution in a properly
labeled glass bottle at refrigerated temperatures (~2-4ºC) and prepare a fresh set of standards every 6 months.

Intermediate Working Standards Preparation

Partially fill five 100 mL glass volumetric flasks to a few centimeters below the meniscus with ≥18 MΩ-cm water.
Add the appropriate volume (Table 7) of each intermediate stock solution into the five flasks. Mix these well,
then dilute each to a final volume of 100 mL with ≥18 MΩ-cm water. Different volumes can be prepared by
spiking with proportionally smaller or larger additions of components.
The final concentrations of iodine in each of the intermediate working standards can be calculated by the formula below. The values entered into the ICP-DRC-MS software should be the concentrations of the intermediate working standard.

\[
\text{Working Standard Conc. } \mu\text{g/L} = \frac{\text{Int. Stock Std. Conc. (µg/L)} \times \text{Int. Stock Std Spike (L)}}{0.100 \text{ L}}
\]

Mix standards well and allow to equilibrate. If time allows, test one aliquot of the calibrators before aliquoting into labeled glass bottles for storage with bench QC and reference materials (as available) before using it for patient sample analysis. Store at refrigerator temperatures (~2-4°C). Expiration date is 6 month from preparation.

**Working Standards (Calibrators)**

The working calibrators are dilutions of the five intermediate working standards into a urine matrix (base urine) for the purpose of a matrix-matched external calibration of an analytical run (run calibrators). Prepare the working calibration standards along with patient samples and QC using the same diluent solution. Use the same base urine for all calibrators and urine blanks to be used within the run. To prepare the working calibration standards, transfer 500 mL of the appropriate aqueous intermediate working standard, 500 mL of base urine, and 4,000 mL of diluent to a 15 mL PP centrifuge tube by using the Micromedic Digiflex™. Cap the tube and mix well before analysis.

### Table 7. Intermediates working standards preparation

<table>
<thead>
<tr>
<th>Name</th>
<th>Intermediate STD (IOD-B)</th>
<th>Intermediate STD (IOD-A)</th>
<th>Concentrations µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD 1</td>
<td>80</td>
<td>240</td>
<td>8</td>
</tr>
<tr>
<td>STD 2</td>
<td>240</td>
<td>80</td>
<td>24</td>
</tr>
<tr>
<td>STD 3</td>
<td>800</td>
<td>240</td>
<td>80</td>
</tr>
<tr>
<td>STD 4</td>
<td>240</td>
<td>240</td>
<td>240</td>
</tr>
<tr>
<td>STD 5</td>
<td>800</td>
<td>800</td>
<td>800</td>
</tr>
</tbody>
</table>
**Range of Linearity**

Partially fill three 100 mL glass volumetric flasks to a few centimetres below the meniscus with ≥18 MΩ·cm water. Pipette the appropriate volume (Table 8) of each intermediate stock solution into the three flasks. Mix these well, then dilute each to a final volume of 100 mL with ≥18 MΩ·cm water. Mix standards well and allow to equilibrate. If time allows, test one aliquot of the calibrators before aliquoting into labeled glass bottles for storage. Test the solutions to verify the concentrations with bench QC and reference materials (as available) before using for patient sample analysis. Store at refrigerator temperatures (~2-4°C). Expiration date is 6 months from preparation.

<table>
<thead>
<tr>
<th>Range of Linearity (RLT)/Calibration Verification (CV) Intermediate Working Standards Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLT/CV working standards number</td>
</tr>
<tr>
<td>RLT / CV 1</td>
</tr>
<tr>
<td>RLT / CV 2</td>
</tr>
<tr>
<td>RLT / CV 3</td>
</tr>
</tbody>
</table>

**Sample Preparation**

Thaw the frozen urine specimens; allow them to reach ambient temperature (about 20°C). Set up a series of 15 mL PP centrifuge tubes corresponding to the number of blanks, standards, QCs and patient samples to be analyzed. Prepare the following solutions into the 15 mL PP centrifuge tubes by using the Micromedic Digiflex™ (Table 9).

<table>
<thead>
<tr>
<th>Preparation of Samples for Analysis (All Volumes in µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID</td>
</tr>
<tr>
<td>Urine Blank</td>
</tr>
<tr>
<td>Calibration STD</td>
</tr>
<tr>
<td>Aqueous blank</td>
</tr>
<tr>
<td>Urine sample or QC</td>
</tr>
<tr>
<td>2x dilution of urine sample*</td>
</tr>
</tbody>
</table>

These volumes are used because the total volume each sample this method consumes is around 3,000µL.

* Volumes listed here are an example of how to combine the correct proportions of water, urine and diluent in making a 2x dilution. Other volumes of the same proportions can also be used. Other dilutions can be prepared as needed by adjusting the proportion of urine to the total volume of diluted sample. Use pipettes at greater than 10% volume capacity for best accuracy.
Prepare an aqueous blank that consists of 1,000 mL of ≥18 MΩ·cm water and 4,000 mL diluent. Use the aqueous blank for the QC pools and patient samples.

Prepare five urine blanks that consist of 500 mL of base urine (same material used for preparation of the urine calibration standards), 500 mL of ≥18 MΩ·cm water, and 4,000 mL of diluent. Run one of these as the blank for the calibration standards. Run two urine blanks after standard 5 (as sample IDs UrBlkChk1 and UrBlkChk2, respectively). Analyze two urine blanks before the calibration blank to condition the system.

Prepare the working calibration standards as described in page 57.

Prepare dilutions (10x) of the QC and patient urine samples using manual or automated pipettors: 500 mL of ≥18 MΩ·cm water, 4,000 mL of the diluent and 500 mL of the patient or QC urine sample.

Cap all of the blanks, standards and samples; mix them well.

It may be necessary to operate the instrument with cell gas flowing at the method flow rate for at least 30-45 minutes before the run begins to allow conditions in the reaction cell to equilibrate; this may be done by analyzing the rinse solution for 8-10 sample cycles prior to analysis of the first conditioning blank.

Note: The cell gas will automatically turn off after 1 hour of no ICP-MS DRC mode analysis.

Uncap and place the dilution preparation of the blanks, standards, QC and patient samples in the autosampler of the ELAN® ICP-DRC-MS immediately prior to start of the analysis run.

Limit of Detection

The LOD for iodine in urine specimens is based on three times the standard deviation of approximately 20 or more measurements of urine blanks or low concentration urine samples, each analyzed in a separate run. This represents the method detection limit. Report results below the detection limit as “< LOD” (where “LOD” is the calculated lowest detection limit). The LOD calculation may be reevaluated annually.

Reference to any commercial entity or product or service in this booklet should not be construed as an endorsement by the Government of the company or its products or services.
Current Program Participants

### National Laboratories

- **USA**
  - The University of Iowa; Doctor's Data INC; Brooke Army Medical Center; ZRT Laboratory; Centers for Disease Control and Prevention; Mayo Clinic; Boston Medical Center

### International Laboratories

- **Albania**
  - Institute of Public Health
- **Australia**
  - Institute of Clinical Pathology and Medical Research; Pacific Laboratory Medicines Services; PathWest Laboratory Medicine; Canberra Hospital, ACT Pathology
- **Bangladesh**
  - International Centre for Diarrhoeal Disease Research; Institute of Nutrition and Food Science
- **Belgium**
  - Centre Hospitalier Universitaire Sainte-Pierre; RP Lab; Erasme Hospital
- **Bulgaria**
  - National Centre of Hygiene, Medical Ecology and Nutrition
- **Cameroon**
  - National Nutrition Centre
- **Canada**
  - Centre de Toxicologie/INSPQ; Health Canada, Nutrition Research Division
- **China**
  - China National Reference Laboratory for Iodine; Tianjin Medical University; Tianjin Medical University; Xiamen Center for Disease Control and Prevention; Institute of Endemic Disease Control; Fujian Centers for Disease Control and Prevention
- **Denmark**
  - Aalborg Hospital, Department of Endocrinology
- **Ethiopia**
  - Ethiopian Health and Nutrition Research Institute
- **France**
  - CHU de Grenoble
- **Georgia**
  - Imereti Zonal Laboratory
- **Germany**
  - Medizinisches Labor Bremen
- **Greece**
  - University Hospital of Patras
- **Guatemala**
  - Institute of Nutrition of Central America and Panama (INCAP)
- **Hong Kong**
  - Prince of Wales Hospital
- **India**
  - All India Institute of Medical Sciences; Indian Council of Medical Research
- **Indonesia**
  - Laboratorium Biotechnology / GAKY Diponegoro Medic
- **Iraq**
  - Nutrition Research Institute
- **Palestine**
  - Palestinian Central Public Health Laboratory
- **Italy**
  - Agenzia per l'Ambiente; Laboratorio Analisi; National Health Institute; Agenzia Regionale per la protezione dell'Ambiente
<table>
<thead>
<tr>
<th>Country</th>
<th>Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>Hitachi Chemical Co., Ltd.</td>
</tr>
<tr>
<td>Kazakhstan</td>
<td>The Kazakh Academy of Nutrition</td>
</tr>
<tr>
<td>Kenya</td>
<td>Kenya Medical Research Institute</td>
</tr>
<tr>
<td>Kosovo</td>
<td>National Institute of Public Health of Kosovo</td>
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<tr>
<td>Kyrgyzstan</td>
<td>Bishkek City Endocrinological Dispansary</td>
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<tr>
<td>Luxembourg</td>
<td>Laboratorio National de Sante</td>
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<tr>
<td>Macedonia</td>
<td>Institute of Patophysiology and Nuclear Medicine</td>
</tr>
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<td>Malaysia</td>
<td>Kota Kinabalu Public Health Laboratory; Institute for Medical Research; Makmal</td>
</tr>
<tr>
<td>Mongolia</td>
<td>Public Health Central Laboratory</td>
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<tr>
<td>New Zealand</td>
<td>Auckland Healthcare Services; Canterbury Health Laboratories Ltd</td>
</tr>
<tr>
<td>Pakistan</td>
<td>The Aga Khan University</td>
</tr>
<tr>
<td>Papua New Guinea</td>
<td>University of Papua New Guinea</td>
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<tr>
<td>Peru</td>
<td>Universidad Peruana Cayetano Heredia; National Institute of Health</td>
</tr>
<tr>
<td>Philippines</td>
<td>Food and Nutrition Research Institute</td>
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<td>Romania</td>
<td>Institute for Mother and Child Care Alfred Rusescu</td>
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<td>Russia</td>
<td>National Research Center of Endocrinology</td>
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<td>Senegal</td>
<td>Centre Hospitalier National d'Enfants Albert Royer</td>
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<td>Serbia</td>
<td>Institute of Public Health of Serbia</td>
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<tr>
<td>South Africa</td>
<td>Nutritional Intervention Research Unit, Medical Research Council</td>
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<tr>
<td>South Korea</td>
<td>Yonsei University, Gangnam Severance Hospital</td>
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<tr>
<td>Spain</td>
<td>Departamento de Sanidad Gobierno Vasco, Bilbao</td>
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<tr>
<td>Sri Lanka</td>
<td>Medical Research Institute</td>
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<td>Switzerland</td>
<td>Laboratory for Human Nutrition</td>
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<td>Taiwan</td>
<td>Taipei Veterans General Hospital</td>
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<td>Tanzania</td>
<td>Tanzania Food and Nutrition Centre</td>
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<tr>
<td>Thailand</td>
<td>Ramathibodi Hospital; Institute of Nutrition &amp; Mahidol University; Research Institute for Health Sciences; Siriraj Hospital; Neonatal Screening Operational Centre</td>
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<tr>
<td>Turkey</td>
<td>Hospital Ibni Sina Hastanesi; Tubitak Marmara Research Center</td>
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<tr>
<td>Ukraine</td>
<td>Institute of Endocrinology &amp; Metabolism</td>
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<tr>
<td>United Kingdom</td>
<td>Biolab Medical Unit; Kings College Hospital</td>
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<tr>
<td>Uzbekistan</td>
<td>Republican Institute of Endocrinology</td>
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<tr>
<td>Wales</td>
<td>University Hospital of Wales</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>Ministry of Health and Child Welfare</td>
</tr>
</tbody>
</table>
The Challenge of Iodine Deficiency Disorder

EQUIP 10 year anniversary
Acknowledgements

One of the greatest benefits of our program is the high level of confidence that participating laboratories have in the results they obtain while conducting their urinary iodine procedures. This confidence is delivered through the work of many dedicated individuals, whom the authors would like to now recognize.

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E-mail address: iodinelab@cdc.gov
Website: http://www.cdc.gov/labstandards/

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