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GENERAL PAPER

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EQUIP: a worldwide program to ensure the quality of urinary iodine procedures

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Abstract In 2001 the Centers for Disease Control and Prevention (CDC) established a program, Ensuring the Quality of Urinary Iodine Procedures (EQUIP); to assist laboratories around the world and assess the accuracy of their urinary iodine (UI). CDC designed EQUIP to issue unknown specimens to participating laboratories three times per year. Each laboratory was asked to analyze unknown samples in duplicate on three different days. During the first five rounds of EQUIP, 41 laboratories participated, measuring unknown samples and reporting their results to CDC. CDC used these results to prepare a statistical report for the laboratories. Feedback to the laboratories provided external confirmation regarding performance. As a group, laboratory performance improved; several laboratories made considerable improvement. Several laboratories that showed no improvement have ordered new equipment or are arranging for additional training. EQUIP is a key tool used to support laboratory quality assurance in an effort to eliminate iodine deficiency disorders (IDD) in the world.

Keywords Urinary iodine · Iodine deficiency disorders · Laboratory testing · ICP-MS · Laboratory proficiency · Laboratory methods

Introduction

Iodine is an essential component of the thyroid hormones, thyroxin and tri-iodothyronine, which are necessary for normal growth, development, and metabolism during gestation, infancy, and throughout life. Iodine deficiency disorders (IDD) are thought to affect more than a billion people worldwide, and IDD during pregnancy and infancy are a major cause of intellectual impairment and brain damage [1]. Since 1990, progress toward the elimination of IDD has been substantial. Today an estimated 70% of the world's edible salt is being iodized [2, 3].

Urinary iodine (UI) concentrations directly reflect dietary iodine intake and UI analysis is the most common method used worldwide for biochemical assessment of the iodine status. At the time of this study no certified reference materials were available for urinary iodine analysis.[1]

The Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention (CDC) uses Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to measure UI in the US population through the NHANES survey and other studies [4, 5]. Our laboratory also maintains proficiency with iodine spectrophotometric methodologies that are similar to the methods commonly used to measure urinary iodine in laboratories worldwide. Through this endeavor we are able to provide technical assistance if needed. In our laboratory we observed that the iodine method previously promoted by the Program Against Micronutrient Malnutrition (PAMM) laboratory [6] tended to be nonlinear, and results were positively biased at the low end and negatively biased at the high end of the normal-population concentration range, when compared to the ICP-MS method results obtained at CDC (as described previously) [5]. In part, because there were no primary or secondary matrix-matched reference

[1] In (2003) the National Institute of Standards and Technology (NIST) included iodine in its most recently introduced urine multi-element reference material (SRM 2670A). This is the first urine iodine primary reference standard, which should prove to be a useful tool to laboratorians worldwide.

materials available for the measurement of urinary iodine, CDC developed a program, Ensuring the Quality of Urinary Iodine Procedures (EQUIP). This program is designed to provide matrix-matched secondary reference material to laboratories measuring urinary iodine around the world. EQUIP target values are assigned, by CDC using ICP-MS, which provides a high degree of accuracy and precision in the measurement of iodine. In this report we will describe EQUIP and compare experience during the first five rounds of the program.

Methods

EQUIP samples were prepared by the CDC laboratories. Iodine spiking solutions were always traceable to the National Institute for Standards and Technology (NIST). We collected urine through an anonymous collection process into lot screened sample collection cups. We then screened each collected sample for iodine content before mixing together to make separate urine pools. Pools were mixed into acid-washed carboys. We then analyzed a sample of each pre-spiked urine pool. We determined the target concentrations for iodine in all pools. Based on the analysis results of the pools and the desired target concentrations, we spike pools using NIST traceable single element stock standard if necessary, to obtain concentrations ranging from about 20–400 μg/L. We mixed pools well after spiking, then reanalyzed. Urine pools were stored for long term in small volumes (1.8 mL) at $\leq -20^{\circ}$C. Storage containers were lot screened and labeled. Dispensing was accomplished using a Digiflex automatic pipettor in continuous cycling dispense mode. Urine was mixed well during dispensing using an acid washed Teflon^{TM} stir bar and large stir plate. After dispensing, we checked homogeneity of analyte concentrations in pool aliquots by analysis of every 20th sample dispensed. We were careful to keep samples pulled for homogeneity analysis in the sequence that they were dispensed for the purpose of looking for trends in concentrations. Once dispensed and homogeneity was shown to be good throughout the tubes of a pool, we store tubes at $\leq -20^{\circ}$C and pull tubes out as needed for shipment. It has been shown that samples tightly sealed do not require refrigeration, addition of preservative or immediate determination. They can be kept for months or more, refrigeration is desired to avoid unpleasant odor. Evaporation should be avoided because this will artifactually increase the iodine concentration. Samples can be safely frozen and refrozen [7].

Laboratories measuring urinary iodine worldwide were invited to join EQUIP. Our laboratory and two other laboratories used ICP-MS. Most of the laboratories used spectrophotometric monitoring of the Sandell–Kolthoff reaction with sample digestion accomplished by using either chloric acid digestion or ammonium persulfate digestion (Table 1).

Participating laboratories were asked during each of the five EQUIP rounds to analyze, using their routine method, three to five samples containing different concentrations of iodine (in a range of 10–300 μg/L) and to report the results to CDC on standardized EQUIP forms. Each laboratory reported data for each sample for three runs in duplicate. With this data we were able to evaluate both precision and accuracy. Each laboratory was asked to report the limit of detection (LOD) for its method. Results were returned to CDC so that each individual laboratory could compare its performance (mean and variance) with individual and composite data from all other participating EQUIP laboratories, whose identities were concealed. All laboratories had the option to seek and receive consultation from CDC laboratorians. Figure 1 is a representative sample report sent to participating laboratories. The statistical report allows EQUIP participants to evaluate their results. Laboratory managers may compare their data to either the CDC target values or to method group statistics. They may compare their coefficient of variance with that of other laboratories.

In order to compare laboratories' performance over time, the difference between a participating laboratory's results for a sample and the CDC EQUIP target was converted to a "Q-score", which is plus or minus a fraction of the CDC EQUIP mean. For CDC EQUIP mean values <50 μg/L, a Q-score of 1.0 was 30% of the CDC mean; for mean values 50–100 μg/L, 25%; and mean values >100, 20%. The equation for calculating the Q-score was:

$$Q\text{-score} = (\text{mean participants results} - \text{mean CDC results})/\text{acceptable error},$$

with acceptable error being 0.30, 0.25 or 0.20 of the CDC mean result for the concentration as described. A Q-score of 1 means the laboratory's result is ±30% of CDC EQUIP mean when the mean is <50 μg/L; ±25% when the mean is 50–100 μg/L; and ±20% when the mean is >100. Sample results within one Q-score of the ICP-MS value was reported as acceptable. In addition Z-scores were calculated for each sample from each laboratory by comparing that laboratory's results with the mean of all laboratories (after exclusion of any laboratory result that was 4 standard deviations above the mean of all laboratories). In these ways a laboratories performance could be tracked over time for consistency and improvement.

A sample of the same concentration was submitted to participating laboratories during EQUIP rounds 2, 3 and 4, so that individual laboratory consistency for a single

Table 1 Description of methods used by laboratories participating in EQUIP

Method	Description of method	No. of laboratories
1	Ammonium persulfate digestion; spectrophotometric analysis manual	18
2	Chloric acid digestion; spectrophotometric analysis manual	13
3	All other methods: micro-plate reader, auto-analyzer, dry ashing, and ICP-MS	10

Method Groups	Specimen ID	CDC ICP-MS target value (µg/L)	Mean (all labs) (µg/L)	Standard Deviation (all labs)	Acceptable range (µg/L)	Mean (within method) (µg/L)	Standard deviation (within method)	SE	Lab (X) results (µg/L)	Comment
(1) Ammonium persulfate spectrophotometric manual n=13	Pool 2	28.6	27.8	9.9	20.0-34.5	26.3	5.2	1.4		
	Pool 9	90.5	88.7	11.6	67.9-113.1	89.4	10.6	2.9		
	Pool 19	276.7	262.2	32.5	235.2-319.2	275.2	19.9	5.5		
(2) Chloric acid spectrophotometric manual n=10	Pool 2	28.6	27.8	9.9	20.0-34.5	28.2	13.5	4.3	56.5	↑
	Pool 9	90.5	88.7	11.6	67.9-113.1	90.8	14.2	4.5	102.2	
	Pool 19	276.7	262.2	32.5	235.2-319.2	252.0	33.3	10.5	276.3	
(3) All other methods: micro plate reader, technocon auto analyzer, ICP-MS n=8	Pool 2	28.6	27.8	9.9	20.0-34.5	29.8	11.5	4.1		
	Pool 9	90.5	88.7	11.6	67.9-113.1	85.8	12.2	4.3		
	Pool 19	276.7	262.2	32.5	235.2-319.2	253.6	43.4	15.3		

Fig. 1 Report of an individual EQUIP laboratory, round 5 results. Target value=CDC EQUIP mean. Acceptable range=± 30% of target value when target value <50 µg/L;±25% when target value 50 to 100 µg/L; and ±20% when target value >100 µg/L. ↑= above assigned deviation and, ↓=below assigned deviation

value could be determined. The comparison of performance over time identified the number of laboratories falling within one or two *Z*-scores in relation to the mean values of actual EQUIP rounds 1–5. In addition each lab was evaluated on the basis of the number of times a laboratory had participated the program, i.e., a laboratory entering EQUIP round 3–5 for the first time could be compared with all other laboratories participating for the first time, regardless of which EQUIP round(s) in which they participated.

Results

Overall participation in this worldwide survey was excellent, with 41 laboratories from 26 countries agreeing to work together to create a worldwide network of iodine laboratories representing all continents, including laboratories from academia, government, hospitals, and private industry (Table 2).

Comparative success of individual samples and individual laboratories in the five EQUIP rounds to date are summarized in Fig. 2. Figure 2 shows the percent of laboratories that have a *Z*-score (*Z*) greater than 1 or 2. The white boxes indicate *Z*-score's greater than one, while the dark boxes on the plot above indicate *Z*-scores greater than 2. It is evident that the number of *Z*-scores greater than two tends to decline in rounds 2–5. During the five EQUIP rounds; laboratories had better conformity of means

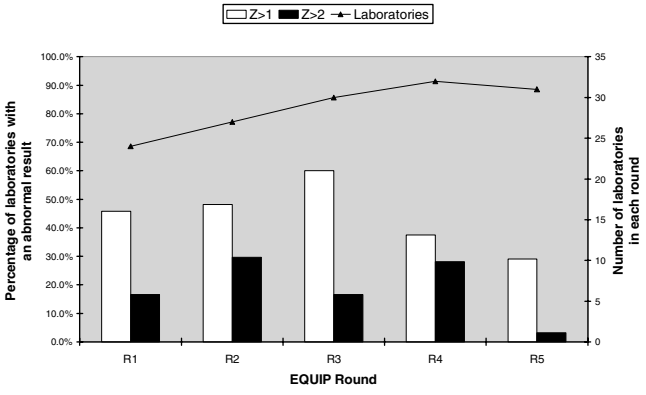

Fig. 2 Laboratories with abnormal results, by EQUIP rounds 1–5

and smaller variance, showing a trend of improvement (Table 3).

These data were reordered by the number of times each laboratory had participated in EQUIP at the time they tested samples and are shown in Fig. 3. Participants are often added or droped out. The laboratories appear to improve as a group with increased number of participating rounds. The coefficient of variance for the ICP-MS method is generally smaller than that of the other methods at all levels tested. There is a slight negative bias at the higher concentrations in the results from the laboratories not using ICP-MS. Since urinary iodine measurements are often used for population surveys as bias, either negative or positive,

Table 2 Distribution and type of laboratories participating in EQUIP

Continent	Type of laboratory				
	Academic	Government	Research institute	Medical	Total
Africa	2	3		1	6
Asia	2	5	3	2	12
Australia-Oceania	1	3		2	6
Europe	2	4		2	8
North America	1	2		1	4
South America	2	2	1		5
Total	10	19	4	8	41

Table 3 Summary, coefficient of variance (CV); analyses of individual samples results, by EQUIP cycle

Note. CV for all runs for all participating laboratories were assembled and analyzed for mean, variance, median, and range for each round

	EQUIP round number				
	1	2	3	4	5
Average CV	7.57	13.07	11.06	8.90	6.34
Standard deviation	5.98	19.01	20.75	7.04	6.34
Standard error	0.55	2.09	1.91	0.62	0.68
Number of samples	119	84	119	131	87
Median	5.68	9.01	5.31	4.91	3.87
Minimum	0.32	0.97	0.47	0.58	0.25
Maximum	35.63	159.87	198.77	63.25	30.18

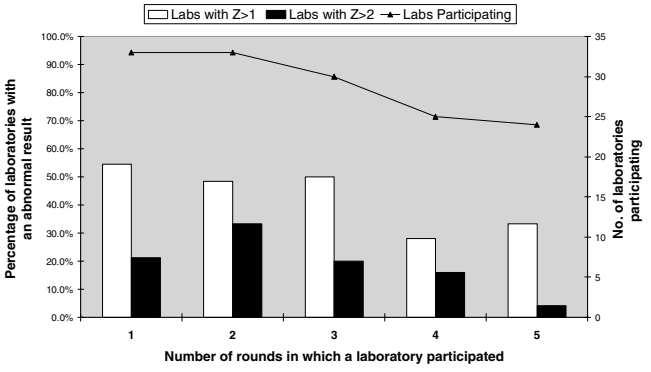

Fig. 3 Individual laboratories with abnormal EQUIP results, based upon number of rounds of participation

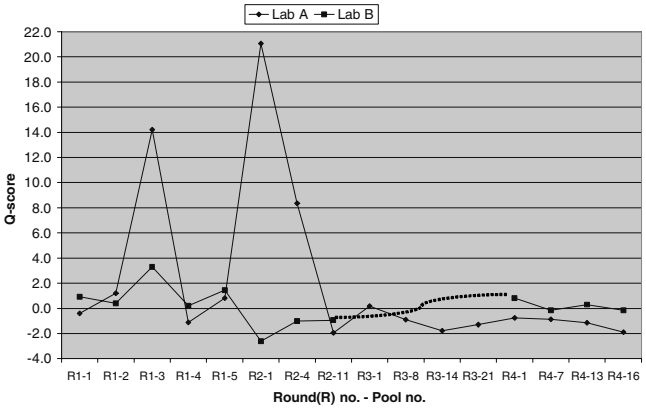

Fig. 4 *Q*-scores for laboratories that improved

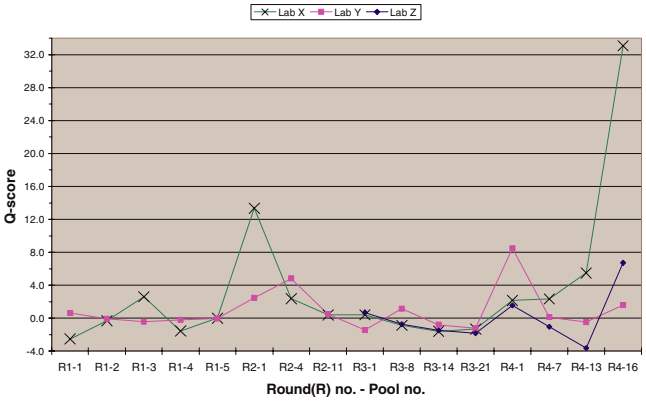

Fig. 5 *Q*-scores for some laboratories showing no improvement, EQUIP rounds 1–4

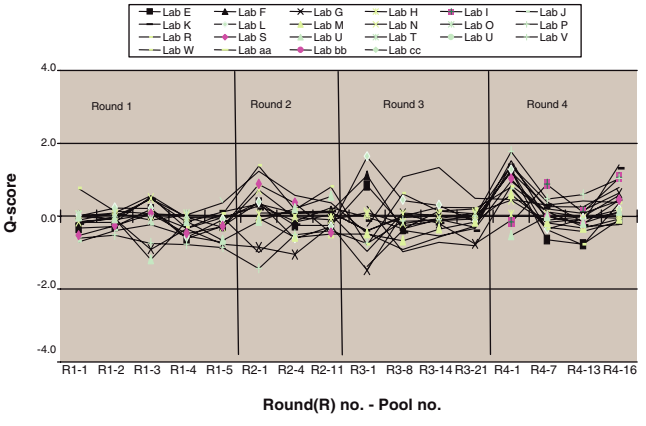

Fig. 6 Laboratories consistently within CDC target values

could contribute to inaccurate reporting of the status of a country's iodine levels. For example, a low estimate of the population median iodine level may lead to salt industry policy changes that could result in over-supplementation. Too little or too much iodine in the diet can have adverse health affects. During the course of the first five rounds, several laboratories changed methods from the chloric acid to the ammonium persulfate digestion method or modified their method for the spectrophotometric analysis. These laboratories often made changes in method on the basis of the EQUIP reports. Some laboratories improved over time with successive rounds of EQUIP, while others showed little or no improvement. Additionally we inserted one sample (Pool 1) three times in EQUIP rounds 2–4. This sample was an additional monitor for round to round consistency and repeatability. Either looking at these three replicate measurements of Pool 1 or the accuracy of all of the submitted samples, some laboratories showed improvement during the five rounds compared with the CDC ICP-MS method (Fig. 4). Some laboratories did not improve (Fig. 5). These laboratories did not show improvement over time regardless of feedback and repeated challenges. Many laboratories consistently reported results within CDC target values. Twenty-three laboratories (66%) reported mean results for all samples analyzed within $\pm 2Q$-scores of the CDC mean (Fig. 6) for all samples. Laboratories using method #1 (ammonium sulfate spectrophotometric manual method) had 98.8% of their sample results within 2*Z*-scores, and 91% within 1*Z*-score of the aggregate mean in the latest two rounds, i.e., EQUIP rounds 4 and 5.

Discussion

The accuracy and precision of measurements of iodine is important not only to monitor the status of iodine nutrition of populations around the world, but it is also important for quality assurance in effort to the eliminate IDD worldwide. For the foreseeable future the ICP-MS method will be used to measure UI samples from NHANES in order to monitor iodine nutrition in the United States, in addition to providing a stable and reliable basis for an external quality assurance program. CDC's EQUIP adds another tool for validation and reference in the process of monitoring the elimination of IDD in the world. The method consistently has small coefficients of variance and thus provides important feedback to laboratories collaborating with EQUIP, as well as a basis for improvement of accuracy and precision over time.

In the 4 years since EQUIP was established in 2001, our data shows the usefulness of this approach, supporting the contention that inter-laboratory comparisons are an effective tool for laboratory performance improvement [4]. There has been significant improvement in the quality of data from laboratories measuring iodine in different parts of the world. All of the methods used by laboratories taking part in EQUIP have demonstrated satisfactory accuracy and precision. Although a few of the laboratories participating in EQUIP reported outlying results more frequently than other laboratories, we believe the data show overall improvement as there was a reduction in unacceptable sample reports and laboratories making such reports. We did not attempt to explain the reported outliers. In previous studies 50% of unacceptable results were attributed to problems in the quality systems, 15% due to clerical problems with new forms, 10% to the study itself, and no cause could be found in 25% of cases [8]. EQUIP was designed to support good laboratory practice and contribute to producing reliable urinary iodine results around the world. Additionally EQUIP was established to fulfill external quality assurance/proficiency testing [9] as required by the Clinical Laboratory Improvement Amendment (CLIA). CLIA requires the following.

1. Adequate training of laboratory personnel.
2. Proper documentation systems for operating procedures, logbooks, records, and reporting.
3. A monitoring system for random and systematic error, using control samples/Levy–Jennings charts and participation in external quality assurance (EQA) programs.
4. Regular internal and external auditing [9].

EQUIP serves to fulfill the need for US laboratories to participate in EQA programs. However, it is questionable whether EQUIP can significantly affect half of the problems that are unrelated to the quality of laboratory practices as described.

In 2001 at a workshop in Bangkok, Thailand, an international group interested in eliminating IDD met and established the International Resource Laboratories for Iodine (IRLI) Network. Twelve of the laboratories participating in EQUIP and representing the World Health Organization (WHO) regions, have been selected to be IRLI Network laboratories to serve as an external monitor for other laboratories that provide clinical and salt-production data for the ongoing efforts to prevent IDD. In November 2002, these 12 laboratories met in Cape Town, South Africa, with sponsoring organizations[2] to develop roles and responsibilities for participating IRLI Network laboratories (Table 4). These laboratories are supported by major organizations around the world and serve as resource centers for laboratories in their regions to provide technical assistance related to analysis of iodine in urine and salt. EQUIP will assist the IRLI Network's development by continuing to provide external quality assurance, collaborating on developing standards of operations, and provide training and assistance as needed to provide an effective system to eliminate IDD [10].

Table 4 Roles and responsibilities of IRLI Network laboratories

- Train personnel and facilitate technology transfer to national laboratories
- Analyze samples where appropriate and possible
- Form regional iodine laboratory networks
- Develop technical standards and external quality assurance/proficiency testing programs within their region
- Collaborate with the salt industry and other sectors on quality assurance and other relevant issues
- Share information with regional networks and communicate with the coordinating body and other interested parties
- Seek necessary resources to sustain the operation of regional networks

Note. IRLI: International Resource Laboratories for Iodine

Conclusions

ICP-MS provides a stable measurement standard by which other laboratories can assess the accuracy and precision when measuring UI. When ICP-MS was combined with EQUIP, the CDC program designed to produce inter-laboratory comparisons, analytical results from participating laboratories improved over time. This system has become a key feature in developing the IRLI Network, a system of support and quality assurance in the effort to eliminate IDD in the world. EQUIP will continue to be a tool available to laboratories performing urinary iodine determinations, providing an assurance of quality data.

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[2] Centers for Disease Control and Prevention, International Council for Control of Iodine Deficiency Disorders, Micronutrient Initiative, UNICEF, and World Health Organization.

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