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Understanding Quality Control with Urinary Iodine Estimation

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Additional information is available at the end of the chapter

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Abstract

Urinary iodine is a tedious assay since it is easily evaporated. A quality system is needed to maintain quality control in a urinary iodine laboratory. In this chapter, a quality system for the urinary iodine micromethod (UIMM) had been discussed briefly. The system covers the pre-analytical, analytical and post-analytical stages of the assay. Each stage depends on each other to complete the whole quality system which ensures the validity of the laboratory results. The laboratory procedures, started with method validation, are very important to be adhered strictly. The internal quality control (IQC) in every analysis and participation in External Quality Assurance (EQA) program will ensure validity of assay and will compare laboratory performance to the others. Evaluation from time to time using Sigma metrics is also vital to complete the quality system as troubleshooting and corrective actions taken will improve the UIMM from time to time. These are supported by the documents and records. A good quality system will guide the urinary iodine analysis operators to gain confidence in their work and the results they obtain for the respondents in monitoring elimination program of iodine deficiency disorders (IDD).

Keywords: urinary iodine, quality control, urinary iodine micromethod, sigma metrics, iodine deficiency disorders

1. Introduction

Iodine deficiency disorders has been one of the targets for elimination by the World Health Organization (WHO) throughout the world. It is a nutritional related disease which is preventable through adequate iodine supplementation. Iodine facilitates optimal brain development

in fetuses and it is involved in the synthesis of thyroid hormones, one of the vital hormones in human body. Hence, with optimum iodine supplementation, it could lead towards more intelligent population.

Urinary iodine is the test in determining the baseline of a population's iodine nutrition before decision of implementing Universal Salt Iodization (USI) is made. It is also important to monitor the iodine nutrition of the population after USI has been implemented. Sampling for urinary iodine testing among school children is non-invasive and urinary iodine is a reliable biomarker for immediate iodine level in one's body. Although thyroglobulin is the biomarker for long-term iodine nutrition in a human, urinary iodine remains the chosen biomarker for the purpose of easier and cheaper way of estimation of iodine nutrition status worldwide. In ensuring the validity of urinary iodine tests results, quality control has to be implemented in the laboratory. In the subsequent sections of this chapter, the quality control plans and implementation are discussed for the benefit of urinary iodine laboratory managers and operators.

1.1. Iodine deficiency disorders (IDD)

Iodine deficiency disorders (IDD) can cause delayed brain development, stunting and stillbirth, and affects humans throughout their life. IDD has been a focus for elimination by the World Health Organization (WHO) since it is a preventable disease through intervention of adequate iodine nutrition. Human residing in the mountain areas are prone to be iodine deficient since iodine is swiped down by rainfall towards the sea [1]. IDD is segregated into severe, moderate, mild IDD. Examples of symptom are goitre, retardation & cretinism (**Figure 1**). The iodine nutritional status of a population is usually determined from median urinary iodine of schoolchildren aged 8–10 years old [2]. Elimination of IDD may reflect the growth of more

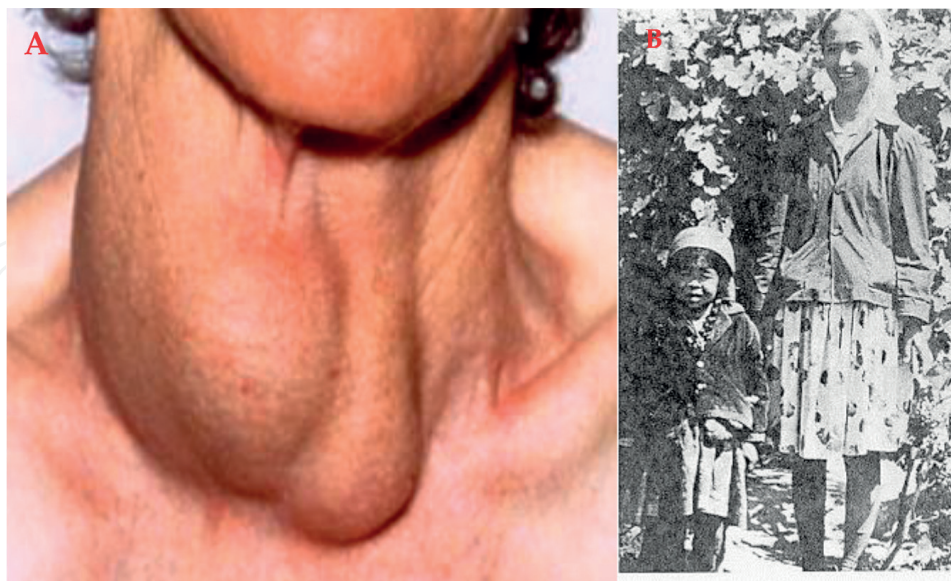


Figure 1. Iodine deficiency disorders (IDD) symptoms, e.g. (A) goitre & (B) cretinism [image reproduced with permission of the rights holders, www.drsosha.com & Human Info NGO; credit is given to United Nations Administrative Committee On Coordination (Sub-Committee On Nutrition)].

intelligent generation to come since it was reported that babies with higher intelligent quotient (IQ) were born from mothers with adequate iodine nutrition during pregnancy [3]. Iodine deficiency may also affect the production of thyroid hormones since each of them need iodine to be covalently bound to the tyrosine backbone [4]. Lack of thyroid hormone production may lead to hypothyroidism and may affect many metabolisms in human body [5]. Thus, iodine is very vital to human growth and development.

1.2. IDD elimination program

IDD elimination program is carried out worldwide. Various interventions of iodine have been implemented including through iodized water and iodized salt. Intervention of iodized salt is the most cost-effective strategy in the elimination program. Iodized salt interventions require only investment of 5–10 cents/year per person [6]. In 20 years, iodization of salt had reduced the prevalence of IDD, whereby, in the year 1993, the number of 131 iodine-deficient had been reduced to only 31 countries in the year 2014. In 2014 also, 70% households had access to adequately iodized salt. Within the years 2009–2013, it was estimated that 50–86% of households are consuming adequately iodized salt, ranging from the least developed countries to East Asia and Pacific countries [7].

1.3. IDD monitoring and determination of median urinary iodine of population

Urinary iodine testing is mandatory before any intervention implementation and in monitoring the universal salt iodization (USI) programs. Urinary iodine laboratories are responsible to perform the urinary iodine testing on respondent samples in determining the population median values. Even though the target group to determine median urinary iodine is school-children aged 8–10 years old, various researches were also done on pregnant women and followed up with their babies to investigate the relationship between iodine status during pregnancy and the babies' IQ [3]. Thus, quality control throughout the laboratory performance is of utmost importance to ensure that the results obtained are valid and reliable to generate accurate reports.

2. Quality in urinary iodine laboratories

2.1. Quality management system

The urinary iodine laboratories (there are five laboratories in the country) were formed under the National IDD Eradication Program parallel with the initiative by the World Health Organization (WHO) for eliminating IDD worldwide. Quality management has been practiced throughout the Ministry of Health following the twelve elements outlined by WHO [8] (**Figure 2**). However, in this chapter, only the quality control aspects related to the urinary iodine laboratory quality management will be discussed, mainly on the processes related to the analysis.

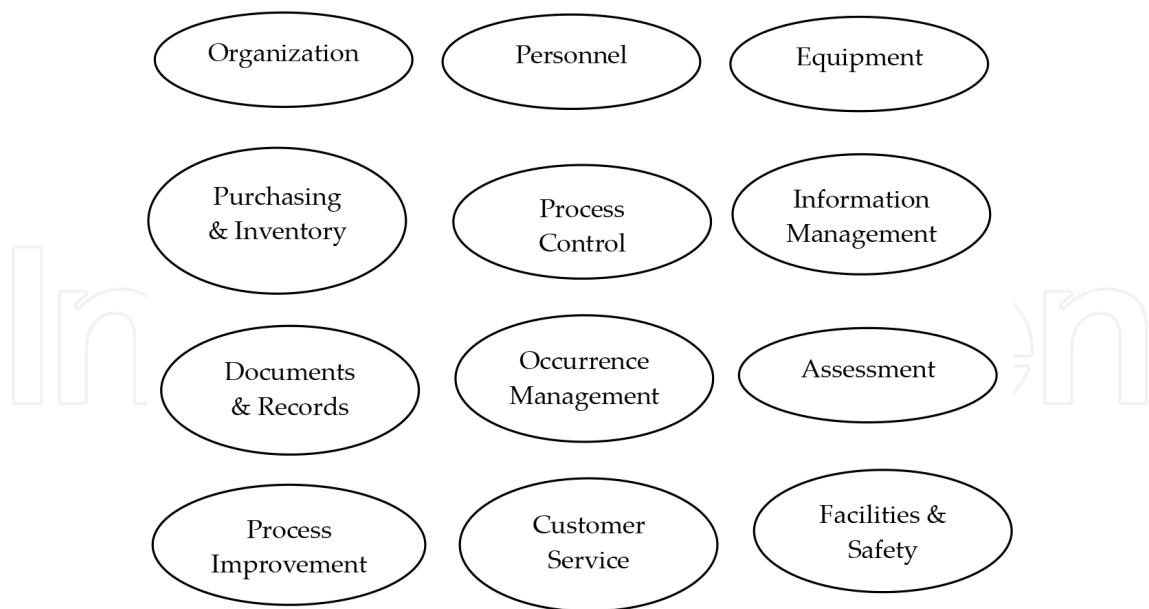


Figure 2. The 12 elements of quality management outlined by WHO [8].

2.2. Quality system in a urinary iodine laboratory

2.2.1. Method

Following the Quality System (**Figure 3**), it started with method modification done in 2004 with migration from performing test wholly in test tubes to performing test half-way in test tubes (during sample digestion) and half-way in 96-well microtiter plates (during reagent mixing and absorbance reading). Method validation was done by the Institute for Medical

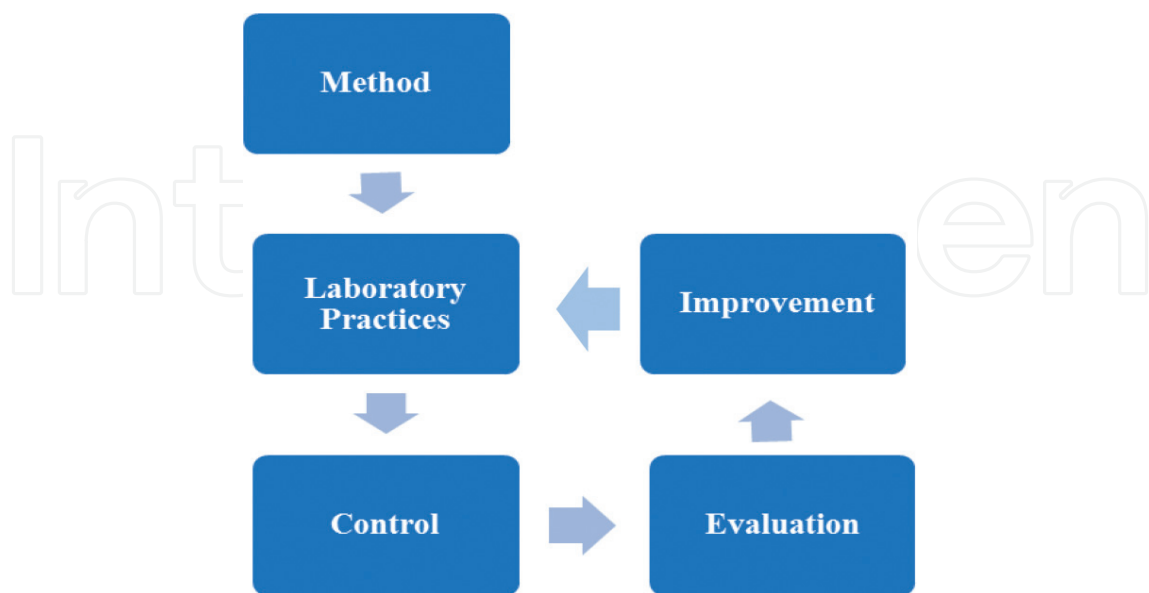


Figure 3. Flowchart of Quality System in the urinary iodine laboratory at the Institute for Medical Research (IMR), Malaysia.

Research from the year 2005 until 2006, comprised of sensitivity, precision, linearity, recovery and method comparison [9].

2.2.2. Laboratory practices

Training is important to enhance skills and competency in performing laboratory work. Maintenance of instruments is vital to ensure adequate heating during sample digestion, correct pipetting of samples and reagents, and accurate absorbance reading. Another important precaution to be made is to avoid contamination from salt iodine laboratory and unclean glassware. Since iodine is easily evaporated, iodine in salt which are usually present in parts per million (ppm) can be dispersed in the air in the same room environment and interfere with urinary iodine measurement which is in parts per billion (ppb). Inadequate cleaning of glassware may cause false detection of high concentration of iodine in urine. Reagents shelf-life should also be abided strictly as aged reagents may cause internal quality control (IQC) values to be out of limits.

2.2.3. Control

IQC sample preparation follows the order of $\%CV \leq 20\%$ for Low control, $\leq 15\%$ for Medium control and $\leq 10\%$ for High control, in obtaining allowable ranges ($\text{mean} \pm 2SD$) from replicates of samples of $n \geq 20$. The order of $\%CV$ set for the laboratory superseded the $\%CV$ set by TUIQP, previous EQA program for urinary iodine which set $\%CV$ of 20% for all Low, Medium and High control ranges. Each control level (Low, Medium or High) should be included in the assay with minimum replicate of $n = 2$. External Quality Assurance (EQA) was done once or twice/year (2006–2009) and is currently being done for three cycles/year (2010–present). Current EQA program provides four concentration levels of samples and requires to be assayed in duplicates in three independent assays ($n = 6$).

2.2.4. Evaluation

Evaluation is performed from IQC and EQA results. IQC results are obtained from every assay while EQA results are obtained from every cycle of the program. Laboratory performance was also determined by conducting evaluation using the Six Sigma quality metrics which focused on the laboratory achievement as compared to the world-class level of Sigma-6 [10]. Evaluation should be done periodically.

2.2.5. Improvement

Corrective actions are made upon every occurrence of non-conformance. Corrective actions are meant to troubleshoot problems and prevent them from being repeated. Relevant IQC rules [11] are to be obeyed and corrective actions are done accordingly to improve quality of test. Improvement may lead to better laboratory practices and the cycle of the Quality System (**Figure 3**) continues as it gets better throughout time.

A well-managed laboratory quality system will enable good laboratory practice, assessment of method, instruments and laboratory performance, and will help the interpretation of respondent results by knowing the accuracy of the method used for Urinary Iodine measurement.

In defining the scope of method, all method validation data should be noted [9] including the expected precision and accuracy and method robustness. The type of equipment to be used as listed in the instrument maintenance section should be noted. The method is applicable to all laboratories possessing the three main instruments, i.e. the heating block, microplate reader, single channel and multichannel micropipette. It is also important that the heating blocks are placed in a fume hood during sample digestion at 100°C for 1 hour so that any fume accumulated can be channeled out from the laboratory for safety purposes. Another vital issue is the skills and competency of the operators which determine the high precision and accuracy of results especially on the pipetting which ensures excellent replicates.

2.3. Urinary iodine analysis

2.3.1. Urinary iodine micromethod

Urinary iodine is a biochemical indicator in monitoring iodine deficiency disorders (IDD) [12]. Urinary iodine is measured using the urinary iodine micromethod (UIMM) which was modified to improve method used in the urinary iodine laboratories in the country [9]. The modified method offers minimal expenditure for new devices, usage of less hazardous chemicals and lesser amount of chemical waste produced. Through method validation, comparison plot and difference plot [11] had been prepared for UIMM against the urinary iodine measurement method proposed by the World Health Organization (WHO) [12]. From the comparison plot (Figure 4), we are ensured that the UIMM works well and it is comparable to the WHO method with excellent correlation coefficient (r) of 0.9428. From the difference plot (Figure 5), the performance of UIMM is shown with not much difference from the WHO method with

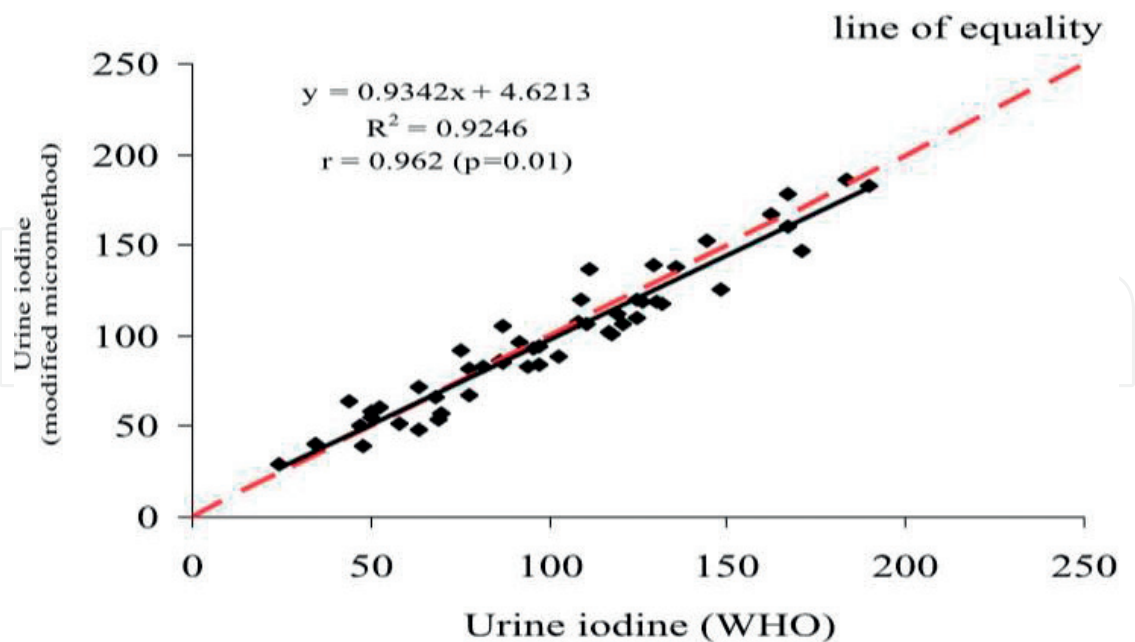


Figure 4. Comparison plot of the modified method versus WHO method (image reproduced with permission of the rights holder, Tropical Biomedicine).

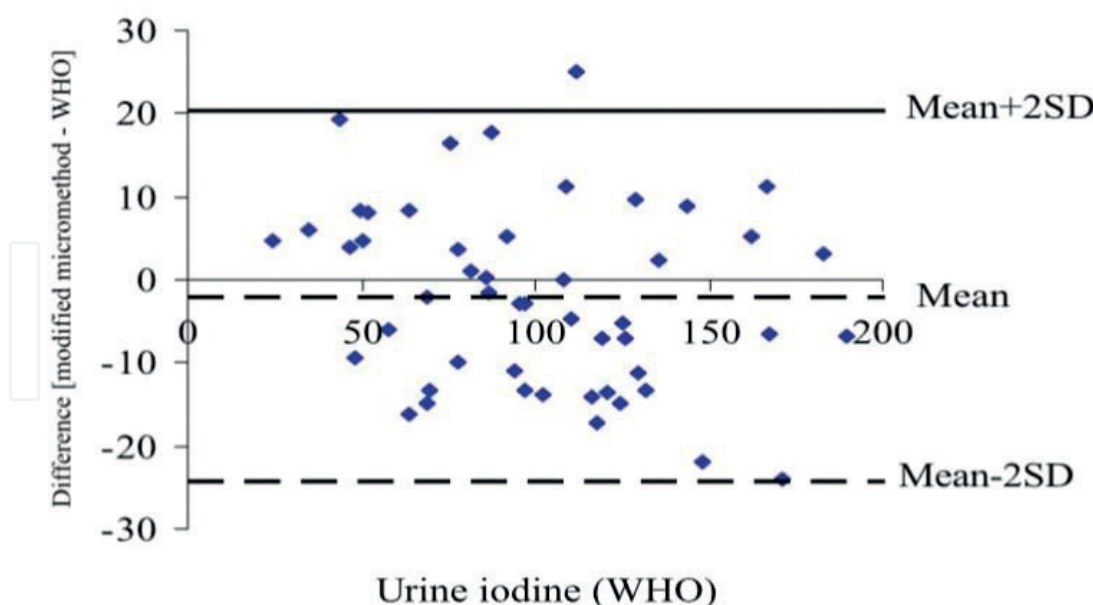


Figure 5. Difference plot between the modified method and the WHO method (image reproduced with permission of the rights holder, Tropical Biomedicine).

only two out of 50 readings with biases of more than $\pm 22 \mu\text{g/l}$. Other method validation includes (i) sensitivity: $13.809 \mu\text{g/l}$, (ii) intra-assay precision: 5–13%, (iii) inter-assay precision: 7–15%, (iv) linearity: correlation coefficient (r) = 0.993, and (v) recovery: 106–114% [9].

Mainly, there are three main solutions used in the UIMM, namely ammonium persulfate, arsenious acid and ceric ammonium sulfate solutions (**Table 1**). The former oxidizes the urine samples and the two latter solutions contribute to the execution of the Sandell-Kolthoff reaction.

The main steps in the UIMM are sample digestion and Sandell-Kolthoff reaction [reaction formulas (1) and (2)]. Urine digestion eliminates the interferences which may cause false positive in the analysis [13]. Arsenite in the presence of iodine reduces yellow-colored ceric ions to colorless cerous ions. Thus, by spectrophotometrical measurement, the absorbance is inversely correlated with the concentration of urinary iodine.

Urinary iodine determination incorporation of two steps of action, i.e. urine digestion at high temperature and iodine measurement in Sandell-Kolthoff reaction of:



The procedural steps for UIMM comprised of four steps as depicted in **Figure 6** [9].

Successful analytical procedures are supported by good pre-analytical (involves documents, chemicals, consumables and glassware) and post-analytical processes (involves records and reports). These processes are discussed further in the subsequent subsections.

Chemicals	Purpose
Ammonium persulfate	Digestion
Arsenious acid solution (As_2O_3 , NaCl , H_2SO_4)	Adding As^{3+} ions
Ceric ammonium sulfate, H_2SO_4	Adding Ce^{4+} ions

Table 1. Purpose of each chemical addition in the urinary iodine micromethod (UIMM).

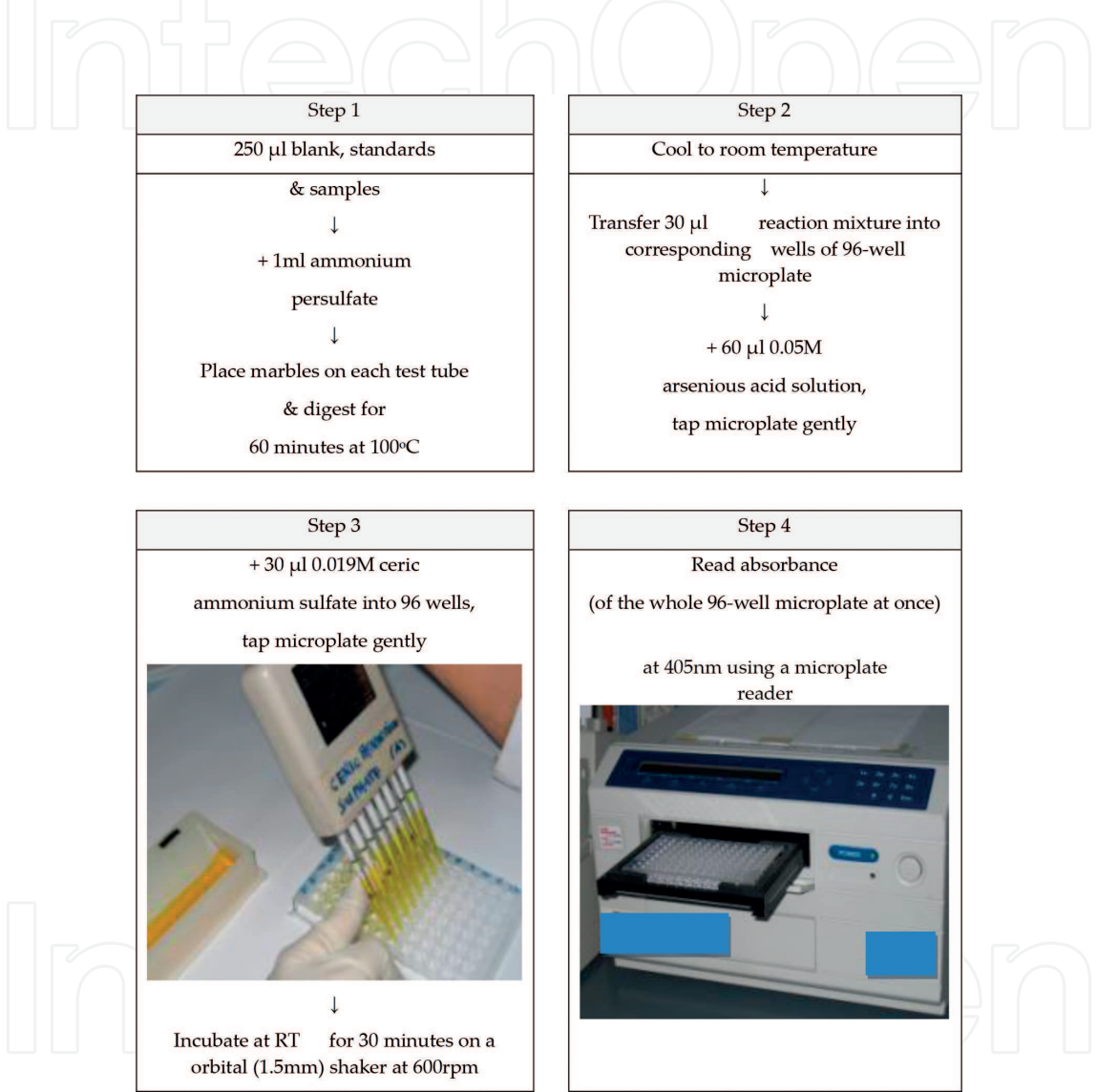


Figure 6. Diagram showing steps in UIMM assay.

2.3.2. Chemicals, consumables and glassware

Chemicals to be used for urinary iodine measurement should be more than or equivalent to analytical reagent-grade. Consumables to be used are non-sterile while the 96-well microtiter plates can be used either of flat- or round-bottom polystyrene ones. Microtiter plate lids should be covered with aluminum foil to prevent direct light onto reaction mixture. Sandell-Kolthoff reaction is sensitive to heat [14]. Thorough washing of glassware (test tubes, volumetric flasks,

beakers, glass marbles) is required to avoid carryover of the leftovers of iodine in the glassware into subsequent assay. Current practice of soaking glassware in distilled water overnight for two consecutive days after washing with detergent is adequate to remove iodine residues and to ensure cleanliness for usage in the next assay.

2.3.3. Maintenance of equipment

2.3.3.1. Heating block

During the digestion procedure of the urinary iodine assay, it is essential that heat be distributed evenly across the heating block on every test tube. Check for even heat distribution can be achieved by placing twenty test tubes filled with 2 ml oil or sand and inserted with a thermometer each. Heating block should achieve 100°C before placing the test tubes and heated for 20 minutes. Temperature of each thermometer should be recorded and mean \pm SD is calculated. The temperatures recorded across the digestion unit should fall within the manufacturer's stated temperature distribution range. CV should be $\pm 5\%$.

2.3.3.2. Microplate reader

The uniformity absorbance reading of the microplate reader maintenance checking is done by pipetting 200 μ l of 1:1500 green food coloring in water in the first row (Row A) of a 96-well microtiter plate. Mean, SD, 1.5SD and CV of the readings are then determined. A scatter plot should be graphed for the individual readings with horizontal lines for mean and mean \pm 1.5SD. The number of readings outside mean \pm 1.5SD is determined and its percentage is calculated. Percentage error should be $\leq 20\%$. If it is not achieved, the maintenance check should be repeated and a request for calibration or repair should be lodged if problem persists. Maintenance check up every three-monthly has to be performed.

2.3.3.3. Micropipette

To check the micropipette performances, a maintenance-check-up every three-monthly is performed. Three points of volume should be tested, i.e. within the lowest, middle and highest ranges. For example, if the micropipette volume range is 100–1000 μ l, then the pipette should be checked at 100 and next time around at 500 or at 1000 μ l; it is up to the operator to decide. Water with the chosen volume should be pipetted into 10–20 clean disposable test tubes (LP3 or LP4 tubes). The weight of the tubes with and without water is recorded accordingly. Other information that should be recorded includes the brand and model of pipette, its code number, date of maintenance check-up and name of the person carrying it out. Mean, standard deviation (SD) and coefficient variation (CV) of the readings are determined. Inaccuracy is also determined as follows:

- % Coefficient of Variation (CV) = $\frac{SD}{Mean} \times 100\%$
- % Inaccuracy = $\frac{(\text{calculated mean} - \text{set volume})}{\text{Set Volume}} \times 100\%$

The maintenance check-up is repeated if the CV is $>5\%$ and inaccuracy is $>10\%$. If the problem persists, request for instrument check-up and calibration should be lodged for further action.

2.3.4. Management of documents and records

Documents are communicators of quality system. They cover three main components, i.e. the policies, the processes and the procedures. The policies are basically about 'what to do', processes tell us about 'how it happens' while procedures explain 'how to do it' [15]. These components are communicated through quality manuals, standard operating procedures, working instructions, external documents and job and personnel-related documents.

On the other hand, records are information produced from the laboratory. Among all are forms, charts, test worksheets, patient records and reports, and quality control performance data [16].

Herewith are the documents we have in our Urinary Iodine laboratory:

- i. List of documents for laboratory personnel
 - a. Work norms for Urinary Iodine laboratory staff
 - b. Checklist for Urinary Iodine laboratory staff
 - c. Daily work list for Urinary Iodine laboratory staff
 - d. List for work order, responsibility and relationships for Urinary Iodine laboratory staff
 - e. Annual work target for Urinary Iodine laboratory staff
 - f. Job description for Urinary Iodine laboratory staff
 - g. Summary of job responsibilities for Urinary Iodine laboratory staff
- ii. List of documents for internal quality control
 - a. Levey-Jennings chart for Low control
 - b. Levey-Jennings chart for Medium control
 - c. Levey-Jennings chart for High control
 - d. Worksheet for IQC ranges determination
- iii. List of forms
 - a. Test request form
 - b. Sample rejection form
 - c. Test report form
 - d. Worksheet for Urinary Iodine testing
 - e. Non-conformance and corrective action form
- iv. List of instrument maintenance record forms
 - a. Heating block maintenance record form
 - b. Microplate reader maintenance record form
 - c. Micropipette maintenance record form

- v. List of other related documents
 - a. List of chemicals used for Urinary Iodine testing
 - b. List of SOP, WI and external documents
 - c. Procedural flow chart for Urinary Iodine testing
 - d. Procedural steps for preparation of Urinary Iodine standards
 - e. Main list of Urinary Iodine quality records
 - f. Urinary Iodine standard preparation diagram
 - g. Sample receipt record book
 - h. Record book for results release through telephone
- vi. List of standard operating procedures (SOP)
 - a. SOP for Urinary Iodine testing
 - b. SOP for maintenance of heating block
 - c. SOP for maintenance of microplate reader
 - d. SOP for maintenance of micropipette
 - e. SOP for method validation
 - f. SOP for preparation of IQC samples
- vii. List of working instructions (WI)
 - a. WI for sample collection, storage and transportation
 - b. WI for sample rejection
 - c. WI for sample disposal
 - d. WI for glassware cleaning

Herewith are the records we have in our Urinary Iodine laboratory:

- i. Pre-analytical stage
 - a. Sample receipt (Test request forms)
 - b. Sample rejection forms
 - c. Instrument maintenance reports
 - d. Internal quality control value determination reports
- ii. Analytical stage
 - a. Test worksheets

- b. Instrument print-outs
 - c. Test results
 - d. IQC performance reports (Levey-Jennings chart)
- iii. Post-analytical stage
 - a. Test reports
 - b. External quality assurance/proficiency testing reports
 - c. Non-conformance reports (NCR)
 - d. Management review meeting (MRM) minutes

In management review meeting, the performance of the laboratory is discussed. Source of problems is identified and corrective actions suggestions from staff are noted for further actions.

3. Establishing internal quality control

3.1. Preparation of in-house internal quality control (IQC)

IQC samples are used to verify the validity of laboratory results. Correct results for IQC obtained in an assay give the confidence that the patients' results are correct. The IQC samples are assayed as part of the analysis, together with the standards and patients' samples. The matrix of the IQC samples should be the same as the patients' samples; in the case of urinary iodine, the matrix is human urine. We use the pooled patient samples since there is no commercial IQC for urinary iodine yet in the market.

Pooled patient samples are usually mixed, aliquoted and kept frozen until use. The advantages of pooled patient samples are:

- The material is inexpensive since they are usually leftovers from the previous assays
- The determination of the concentration ranges is flexible since it can be adjusted accordingly
- Same matrix as human sample

The disadvantages are:

- The preparation of the IQC is time consuming
- The IQC materials can be infectious since there is no screening prior to pooling
- They are often unreliable since there are no preservatives added as stabilizers and their shelf life is often short (around 6 months)

Every time we prepare a new batch of IQC, the same procedures ought to be followed:

- i. Analyze material for at least 20 runs
- ii. Calculate the mean
- iii. Calculate the standard deviation (SD)
- iv. Determine range (mean \pm 2SD)

The records of the IQC concentration range determination ought to be kept and referred to every time after assay. The IQC values obtained in an assay are compared to the mean \pm 2SD values. Then, the IQC plotter charts are drawn (Levey-Jennings chart). Example is as depicted in **Figure 7**. The results should be checked; if the IQC values are within the ranges, the respondents' results are considered acceptable and could be reported. If the IQC values are out of range, the respondents' results are unacceptable for reporting and analytical problems need to be identified and solved. Daily IQC performance is very important in laboratories. It is very crucial to use fresh IQC samples in every assay. The IQC samples should be treated the same as treating the respondent samples.

3.2. Procedural steps in IQC preparation

1. Urinary iodine value of each respondent's urine sample is determined.
2. Urinary samples with the value within the target range are pooled:
 - Low pool (L): 30–90 $\mu\text{g/l}$ (e.g. target to get mean around 60 $\mu\text{g/l}$)
 - Medium pool (M): 110–130 $\mu\text{g/l}$ (e.g. target to get mean around 120 $\mu\text{g/l}$)
 - High pool (H): 200–300 $\mu\text{g/l}$ (e.g. target to get mean around 250 $\mu\text{g/l}$)
3. Urine iodine value of each urine pool is determined.
4. The target values are achieved using mixture of L pool and H pool through formulas 1 and 2 in **Table 2**:
5. Urinary iodine value of each modified urine pools is determined again and if the values are around the target values, the IQC pools are accepted as the new batch of IQC.
6. Urinary iodine values for each IQC are determined for at least 20 times (e.g. duplicates in 10 different assays).
7. After outliers are omitted, mean, SD and range (mean \pm 2SD) are calculated for each L, M and H pools and these ranges are used to determine the validity of test results.
8. Every time after thawing frozen pooled urine, it ought to be centrifuged for 1000 g for 15 minutes, supernatant is then taken and mixed well. Pipette aliquots of 250 μl in 500 μl microcentrifuge tubes and keep at -20°C until use.
9. The IQCs in microcentrifuge tubes are thawed and are transferred into test tubes prior to assay, to be added with ammonium persulfate solution and ready for digestion with blanks and respondent samples.

10. Spike of urine samples with potassium iodate ought to be avoided since it is more unstable as compared to using the endogenous iodine in the urine matrix.

3.3. Monitoring QC performance through Levey-Jennings chart

QC results should be checked every time after an assay. There are some rules to refer to when deciding to accept an assay:

Accept assay when QCs are within 2 SD

Reject assay when any QC exceeds $\pm 3SD$

Reject assay when 2 consecutive QCs exceed ± 2 to 3 SD

Reject assay when difference between 2 QCs exceeds ± 4 SD

QC performance should be reviewed regularly to check the precision and accuracy of the assay. Both the acceptable and unacceptable results should be recorded. Corrective actions taken when QC results are unacceptable should also be recorded. Example of Levey-Jennings chart is as depicted in **Figure 7**.

Example:

Target volume = $V_3 = 250\ \mu\text{l} \times 500\ \text{aliquots} = 125,000\ \mu\text{l} = 125\ \text{ml}$

Initial H pool concentration = $M_1 = 400\ \mu\text{g/l}$

Initial L pool concentration = $M_2 = 70\ \mu\text{g/l}$

Target concentration = $M_3 = 250\ \mu\text{g/l}$

Volume L pool to be added = $V_1 = X$

Volume H pool to be added = $V_2 = Y$

Target volume = $V_3 = 125$

Formula 1: $X + Y = 125$; $Y = 125 - X$

Formula 2: $M_1V_1 + M_2V_2 = M_3V_3$; $M_1X + M_2Y = M_3V_3$

$$M_1(X) + M_2(Y) = M_3(125)$$

$$M_1(X) + M_2(125 - X) = M_3(125)$$

$$M_1(X) + M_2(125) - M_2(X) = M_3(125)$$

$$M_1(X) - M_2(X) = M_3(125) - M_2(125)$$

$$(X)(M_1 - M_2) = 125(M_3 - M_2)$$

$$X = \frac{125(M_3 - M_2)}{(M_1 - M_2)}$$

$$X = \frac{125(250 - 70)}{(400 - 70)}$$

$$= \frac{125(180)}{330}$$

$$= \frac{22,500}{330}$$

$$= 68.18\ \text{ml}$$

$$Y = 125 - X$$

$$= 125 - 68.18$$

$$= 56.82\ \text{ml}$$

Table 2. Calculation to obtain the target values of control samples.

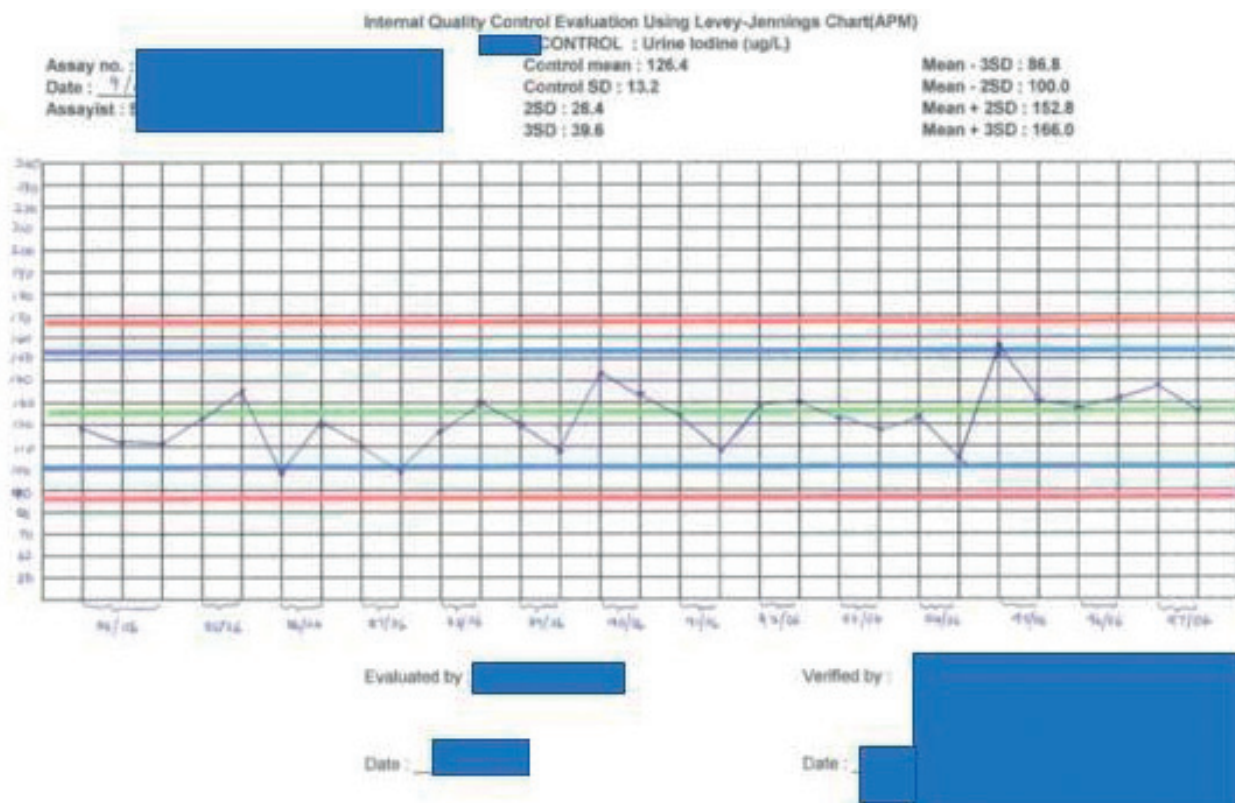


Figure 7. Example of Levey-Jennings chart to monitor IQC performance.

3.4. Non-conformance troubleshooting

If the mean value of the IQC samples is outside the range, the results for respondents in the same range cannot be reported yet and testing should be repeated. The QC charts (Levey-Jennings charts) trends should be checked and the drift in accuracy should be monitored. The cause of drifts should be investigated, e.g. faulty instruments (may cause systematic errors), expired reagents or IQC samples (may cause systematic errors), unclean glassware (may cause random errors) or changes in the laboratory environment (any contamination from iodized salt or elevated temperature may cause systematic errors), or human error (e.g. new operator assigned for the test may cause random errors). The investigation outcome is then comprehended among laboratory personnel and relevant trouble-shooting is taken. The trouble-shooting is recorded as corrective action and it is not a one-time solution to the current problem but also as a preventive step from the problem to occur again in future.

4. Participation in External Quality Assurance program

External Quality Assurance (EQA) is an ISO requirement, to confirm the quality of analysis. It shows the bias and precision of our assay and the position of our laboratory within the same

test method group and against other test method groups. Participation in the EQA program increases confidence of laboratory personnel in performing the analysis.

There are various methods which are under the External Quality Assurance Program, i.e. Sandell-Kolthoff method consists of three different assays: (1) done in tubes, digestion with chloric acid; (2) done in tubes, digestion with ammonium persulfate; (3) done in microtiter plate, digestion with ammonium persulfate. There are other methods such as using the autoanalyzer using dry ashing of urine in potassium carbonate. However, the method with highest sensitivity is the inductively-coupled plasma-mass-spectrometry (ICPMS) but small/medium scale laboratories may not afford to purchase the instrument.

CDC's Ensuring the Quality of Urinary Iodine Procedures (EQUIP) program from the Centre for Disease Control and Prevention (CDC), Atlanta, U. S. A. is worldwide. To date, more than 84 iodine laboratories from more than 50 countries have participated. Our laboratory has participated since the year 2010 until present.

If any urinary iodine laboratories are interested to participate in the EQA program, please visit CDC's website. Application form should be completed and e-mail or fax it to CDC, and a confirmation e-mail will be received within 72 hours. The laboratory will then be enrolled immediately upon receipt of the form and will receive a set of EQA samples every February, June and October each year.

What each laboratory should do is to treat the EQA samples like normal respondent samples and run the EQA samples in duplicates in three assays on different days. Report of the results should be submitted before the deadline within 1 month after receipt of samples.

5. Evaluation of performance of urinary iodine laboratories

Throughout a time-frame, there is need for an evaluation to be done on our urinary iodine laboratory performance. The UIMM had been validated in the year 2006. Since then, all Urinary Iodine laboratories in the country had started using the method. In the year 2008, the National IDD Survey had been carried out [17] and the urinary iodine assays was performed on the respondent samples with inclusion of IQC samples and EQA samples in every assay. The EQA samples were provided by the Institute for Clinical Pathology & Medical Research (ICPMR), Australia. Thus, with the available data, an evaluation on our laboratory performance was performed using the EQA sample results by applying the Six Sigma quality metrics [10]. The higher the sigma metrics the better, and Sigma-6 is the best, depicting very little error or errorless achievements. A method decision chart [11] was plotted for all laboratories (it was four laboratories at that time) and the achievement for every Low, Medium and High controls were determined. The method validation results were also plotted on the chart. There were two plots, i.e. one is set by The Urinary Iodine Quality Program (TUIQP) under ICPMR (**Figure 8**) and the other one is following the limit set by Ensuring Quality in Urinary Iodine Program (EQUIP) (**Figure 9**) under CDC. However, the latter EQA program had been halted

and the former EQA program is the only existing program providing services to various urinary iodine laboratories worldwide. Since urinary iodine is easily evaporated and UIMM uses digested sample of only 30 μl out of 1250 μl of total volume, high %CV is usually high in the lower concentration range of the standard curve. EQUIP which set stricter %CV limit, leads

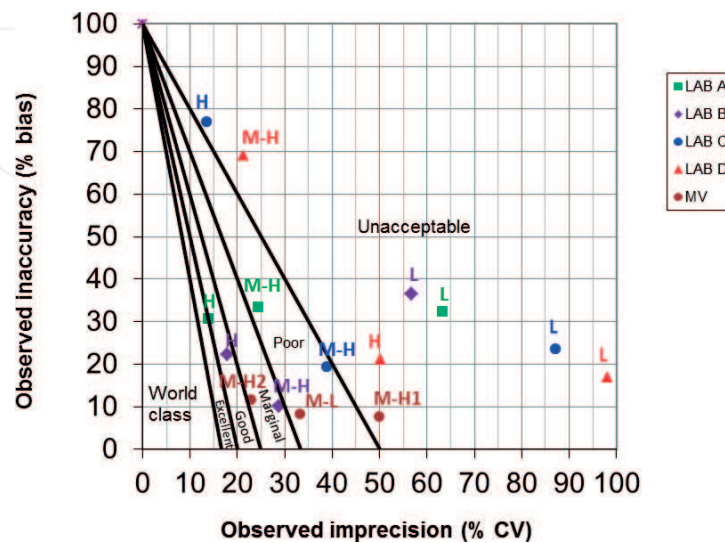


Figure 8. Urinary iodine micromethod's normalized method decision chart against The Urinary Iodine Quality Program (TUIQP) TEAs based on the 2008 National IDD Survey EQA results (Lab A [n = 20], Lab B [n = 18], Lab C [n = 12], and Lab D [n = 6]) and the 2006 method validation study (MV). L indicates low control, M-L indicates medium-low control, M-H indicates medium-high control, and H indicates high control, according to urinary iodine ranges in **Table 1** of reference [10] as reported in Hussain et al. [10] (image reproduced with permission of the rights holder, Annals of Laboratory Medicine).

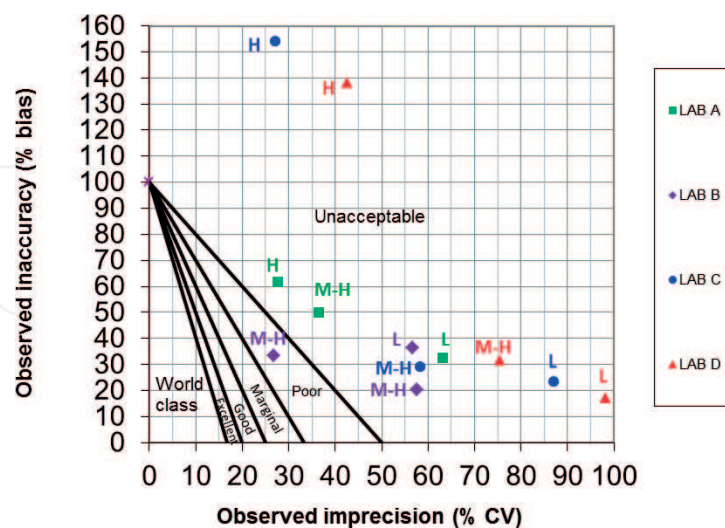


Figure 9. Urinary iodine micromethod's normalized method decision chart based on the 2008 National IDD Survey EQA results (Lab A [n = 20], Lab B [n = 18], Lab C [n = 12], and Lab D [n = 6]) and against EQUIP TEAs. L indicates low control, M-H indicates medium-high control, and H indicates high control, according to urinary iodine ranges in **Table 1** of reference [10] as reported in Hussain et al. [10] (image reproduced with permission of the rights holder, Annals of Laboratory Medicine).

to lower sigma metrics for the Low control (**Figure 9**). High control is easier to pass the %CV limit set by both EQA providers (**Figures 8 and 9**).

Before participating in the EQA program, another way to evaluate the nation’s urinary iodine laboratories was through inter-laboratory comparison. Some analyzed respondent samples were chosen from a wide range of urinary iodine concentrations and the same samples were analyzed again by another urinary iodine laboratory by using the same method. The results were compared between the laboratories and biases were determined. Any discrepancies were then discussed and trouble-shooting to problems was carried out.

6. Safety and waste management

Safety and waste management is very important and included in the quality management system. All Material Safety Data Sheets (MSDS) for every chemical used were printed out and kept in a designated file for reference. The information is used for self-awareness and protection, waste labeling and in any spillage incidences. Wearing proper personal protective equipment (PPE) should be a culture in the urinary iodine laboratory.

6.1. Safety

One of the chemical used in the UIMM is arsenic (III) oxide. It is categorized as highly toxic. Thus, safety precautions ought to be made along the way from purchasing, storage, analytical stages until waste disposal. The safe procedure to weigh arsenic is as discussed below as reference to all operators. Its storage should be in a locked containment with records of its date and time it is being taken out and name of the operators handling it.

6.1.1. Know your urinary iodine chemicals

The properties of UIMM chemicals and precautions that should be taken while handling them are as stated in **Table 3**.

Chemical (properties)	Precaution
Ammonium persulfate (oxidizing substance)	Avoid direct contact to skin or inhalation
Ceric ammonium sulfate (toxic substance)	Avoid direct contact to skin or inhalation
Arsenious acid solution (highly toxic substance) (containing sodium chloride, sodium hydroxide, arsenic (III) oxide and sulfuric acid)	Avoid direct contact to skin or inhalation
Potassium iodate (toxic substance)	The concentration of the working solution is not exceeding hazardous limit but precautions while handling it ought to be taken
H ₂ SO ₄ (corrosive substance)	Avoid direct contact to skin or inhalation

Table 3. Chemical properties and precautions when handling UIMM chemicals.

6.1.2. Weighing of arsenic

1. It is a must to wear R95 or N95 mask while weighing arsenic substances as it is highly toxic and can affect pulmonary system if inhaled accidentally.
2. Prepare two 100 ml beakers:
 - a. one is empty
 - b. one is filled with 20 ml dH₂O
3. Weigh the chemical in the empty beaker
4. Take out the beaker from the weighing scale
5. Pour the water from the second beaker into the first beaker by letting the water flow slowly into the first beaker. This will avoid the chemical from floating into air when transferring the beaker to the work bench.

6.1.3. Other safety precautions

- Do not drain the reagents in the sinks
- Arsenic is highly toxic. Limit of arsenic that can be drained through the laboratory sinks with permit is 0.003 mg/l [18]. Thus, all urinary iodine assay waste should be poured into appropriate waste containers before the glassware is soaked and washed
- Send the waste for disposal properly as discussed in Section 6.2.

6.2. Waste management

- Labels on the waste containers should be legible and clear
- Name of waste and category of waste should be written and printed on the label
- Date of the first time the waste is accumulated and date of the last time the waste is accumulated should be written on the waste label
- A void space in the waste container of approximately 10% should be allowed for expansion
- Waste should be stored in closed containers, placed in a corrosive-proof basin as secondary containment against spillage
- Waste containers for urinary iodine should be placed in the same room but not mixed with wastes from other analysis
- Each reagent waste ought to be placed in individual waste bottle, labeled and dated
- Avoid from putting unbalanced reaction mixture in one waste bottle; this may lead to accumulation of gas and the waste bottle may explode
- Aware of toxicity of reagents.

7. Way forward

7.1. Training

Training of staff running the urinary iodine assays ought to have these goals:

- Achieve competency to do laboratory work
- Understand aspects in the laboratory Quality Plan
- Aware of sensitiveness of urinary iodine test
- Aware of possibility of contamination from iodized salt
- Implement correct waste handling system
- Abide by reagent expiry dates.

7.2. Way forward for small/medium-scaled urinary iodine laboratories

The current reference method for urinary iodine testing is the Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). However, this instrument is most likely unaffordable by small/medium-scaled laboratories due to its high purchasing and maintenance costs. Even though there is high tendency of getting higher bias and deviation when using Sandell-Kolthoff method as compared to using ICP-MS, the small/medium laboratories can still obtain excellent performance by applying a closely-monitored quality management system in the laboratories as discussed in previous sections in this chapter. Decision to participate in the International Organization for Standardization (ISO) 15189 for Quality and Competence for Medical Laboratory will be a plus-point since it consists of all the elements of quality management and ensures quality in the results produced by the laboratory.

8. Conclusion

Even though UIMM, a spectrophotometrical method, is less sensitive compared to the sophisticated methods such as the ICP-MS, the same process of quality system applies to the latter as well. Since urinary iodine is easily evaporated, careful measures have to be made in all pre-analytical and analytical procedures to minimize it. The quality system is supported by detailed documentation and glassware cleaning in the post-analytical procedures. IQC and EQA programs are very important to enhance validity of respondent results to be released to the IDD program managers in monitoring the population iodine status. Urinary iodine estimation is vital to maintain effectiveness of the USI program in eliminating IDD worldwide. This chapter is hoped to be a guide to all urinary iodine laboratories in understanding quality control in urinary iodine estimation.

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Conflict of interest

It is declared that there is no conflict of interest involved in publication of this book chapter.

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