A Method in Field Measurement of Urinary Iodine


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ABSTRACT

The objective of this study is to determine a very cheap, reliable, relatively rapid portable field unit for quantitative urinary iodine measurements. As Individual Urine Excretion (IUE) approximate those of Daily Iodine Intake (DII), iodine deficiency can be measured in casual urine samples of individual. Using the criteria, the frying pot method has been introduced and modified from the long and tedious Zak’s method after Pino et al, and Robbins and Dunn et al. This modified method has many advantages. The assay takes 84 tubes of urine per pot in 100 °C paraffin oil and changes into water. It has been shortened from one hour to 25 minutes. Change of to intermediate violet is observed by adding ferroine as the indicator, resulting in a standard curve reading the concentration of iodine in micrograms per liter. The results of 255 subjects from Mukdaharn Province indicate the reliability of the method as the results are the same as those in the heating block and spectrophotomete. This has been confirmed by the results of 1,007 samples. This new technique provides good results; between oil and water, the results on using water are even better. This modification reduces significantly by lowering the cost of equipment. It is rapid, simple and reliable. The method is portable and valuable for measuring and monitoring iodine deficiency studies and for epidemiological studies of iodine deficiency its control in developing countries.

Keywords: Urinary iodine; Iodine intake; Frying pot method

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Rapidity and portability of urinary iodine measurements are needed in most situations which include the monitoring and evaluation of Iodine Deficiency Disorders (IDD). Monitoring of iodine levels in urine is essential in evaluating the success or failure of prophylaxis in IDD programs. Iodine levels can indicate iodine deficiency or excess; should such conditions be detected, corrective measures can be rapidly introduced.

Because most ingested iodine is excreted in urine, it is possible to estimate daily iodine intake from iodine excretion in casual urine samples. 1-3 In monitoring urinary iodine levels in the field, the Iodine Study Team at the Faculty of Medicine Siriraj Hospital, used a method of on-site testing of urinary iodine samples. The method is useful for population studies en mass, at a significantly lower cost and with speedy results. Using this method, iodine deficiency can be documented quantitatively in micrograms of iodine per day from standard curves of iodine. The results indicate the severity of a public health program (from a joint WHO/UNICEF/ICCIDD definition). The criteria for iodine deficiency includes samples below those of the standard concentration threshold level; <100μg per liter per day is considered mild, less than 50 moderate, and below 20 a severe iodine deficiency. Excess of urinary iodine may also be demonstrated in situations such as oral iodized oil administration in women of reproductive age or an over-dosage of KIO3 as a result of errors in preparing iodized water.

The objective of this study was to evaluate our technique for iodine measurement in urine, which is especially (a) portable (b) rapid and (c) reliable for (d) field use. The new method used water instead of paraffin oil with a short time (25 minutes instead of one hour) in the frying pot with ammonium persulfate before adding of Sandell-Kolthoff® (SK) reagents. Changes in color of the digested solution were noted after adding ferroine.1-7: constructing a standard curve and urinary iodine concentration was read.

MATERIALS AND METHODS

At present, for unknown urine samples, the iodine value can be estimated quantitatively and rapidly. The results are expressed in micrograms of iodine per liter and
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Fig 1. Modified digestion of urine, (a) by the use of a frying pot. "Tefal" in small home-made fume hood, (b) The urine is heated at 100°C for 25 minutes using either paraffin oil or water, which is put in a teflon container around the rows of 84 urine samples. (c) The diagram shows separate parts of the frying pot.

Fig 2. The homemade fume hood illustrates all three pots grouping together with 3x84 samples. Note the broad band of plastic above, preventing the blowing back of the vapor from each frying pot.

compared favorably by using either the portable frying pot or the gold standard method of a heating block and spectrophotometer.

Assay performance of the good method for urinary iodine analysis should have satisfactory results; as the assay performance can be performed as follows

a. Minimal Detection Concentration (MDC) or sensitivity of the assay- 20 blanks (zero point) run in assays (n=20). MDC is calculated as the concentration corresponding to two standard deviations below the mean of time of color change in replicates of the zero point.

b. Analytical recovery 5, 12.5, 20 ng iodine added, in duplicate, to urine samples prior to digestion (n=10). Recovery was calculated as the amount of added iodine measured, expressed as a percentage of the amount of iodine added.

c. Precision - Intra (within) assay: replicate samples run in 1 assay. - Inter (between) assay: duplicate samples in 10 or 15 assays.

In our technique the samples were digested with oil and changed into water which resulted in a shorter time to complete the reaction. Recently, two hundred and fifty five subjects from Mukdahan Province, Thailand, were measured and confirmed by 1,007 studies of urinary iodine using two methods:

(I) the cheaper frying pot method, and
(II) the conventional gold standard heating block-spectrophotometer.

(I). Modified and cheap method using the portable frying pot

In this modified frying pot method, urine samples were digested for 25 minutes with ammonium persulfate. After adding Sandell-Kolthoff (SK) reagents and ferroine, the developed color was observed for change against time and a standard curve was constructed. The procedure of digestion was by the use of a frying pot "Tefal" (Fig 1). A paraffin oil bath was later changed into a water bath in a home-made hood, avoiding the use of expensive standard heating blocks, spectrophotometer, and an elaborate perchloric fume hood.

The frying pot "Tefal" is a domestic appliance, consisting of two overlying containers. The lower one is coated with teflon (polytetrafluoroethylene) and filled with water instead of paraffin oil. The upper one is a sieve with a handle and contains a rack of 84 test tubes of urine. The pot also has a perforated lid and filter, which were not used in this application. Accessories such as power, time and heat control (the temperature is measured separately in the back), are assembled in the front.

A battery of three frying pots is housed in the home-made fume hood in which the three compartments are covered vertically above with a broad band of polyethylene to prevent the blowing back of the contaminating vapor from within the hood. The fume hood is shown in Fig 2.

Procedure

A) Two hundred and fifty microliters (μl) of casual urine samples and the standard were put into tubes, each containing 1 ml of 1M ammonium persulfate and digested in oil alternated with water at 100°C for 25 minutes (in stead of the conventional one hour) in the frying pot. After digestion, the procedure continued as follows.

B) 100 μl of 10% ceric sulphate were dissolved in 10% H₂SO₄ and 100 μl of 7 M NH₄Cl were mixed in one bottle. Besides, 25 μl of 0.5N acetic acid were added to acidify the solution. This addition of acetic acid comes from past experience and teaches us to discriminate the color of paper.

C) One hundred and fifty μl of 5% sodium arsenite and 150 μl of ferroine were put in another bottle.

The reagents in both bottles of SK reagents (B and C) were then mixed and added to the digested urine (A) and the color changed from blue to violet (before the final orange). The time of change to an intermediate violet was considered critical to construct the standard curve.
Preparation of standard solutions for construction of the standard curve:

In the modified method described in this paper, 0.5 g/ml standard KIO₃ stock or urine (A) was diluted to 250 l with the following concentrations: 0, 20, 40, 80, 120, 160, 200 μg/L/tubes after which one ml of ammonium persulfate was added to each tube. The tubes were then digested in the frying pot and added to the previously described reagents - (B) and (C). Finally, ferroine was added to the tubes which indicated different degrees of change of color from a faded blue to the critical violet, (finally to orange). Data regarding concentrations were plotted on graph paper.

II. Gold standard method

In this standard method, a heating block and spectrophotometer were used; ferroine was omitted. Ammonium persulfate was used in the digestion with the heating block, the SK reagents were added, and a laboratory spectrophotometer was chosen to measure optical density.

In the gold standard method, standard KIO₃ concentrations are likewise prepared as above but a spectrophotometer is used to measure the fading of the original yellow color up to the iodine quantity as optical density (O.D.). Having compared the O.D. with various KIO₃ concentrations, the standard curve was plotted on graph paper (Fig 3c).

RESULTS

All results of the modified methods compared favorably with those of the original method. This point is important since it indicates that the precision of all current results are confirmed. Past studies have shown that urinary iodine measurements from the modified Pino method agree with those of the perchloric acid method. A correlation coefficient (r) for the urinary iodine measurements between ammonium persulfate and perchloric acid digestion was 0.994 (n=110, Pino⁸) whereas those between the modified Pino method and the perchloric acid digestion method had r = 0.898 (n= 845, Suansawan¹), respectively. Again, in the ammonium persulphate method, there was a high correlation between the digestion time of 25 minutes and one hour (r= 0.927, n=1,290); therefore, the shorter time of 25 minutes of digestion was chosen for economic reasons. Neither was found to give false positive or false negative results.

Some years ago we used Zak’s method 10 in which a hot plate was used, but it was changed into a heating block technique to prevent evaporated corrosive digestion. Perchloric acid was changed to ammonium persulfate to prevent potential explosions and has been in use until now. All changes are supported as compatible with the current method.

Assay performance of the frying pot method using paraffin oil and water

In Table 1, Minimal Detection Concentration (MDC, μg/L), n=20 of the paraffin oil using the frying pot method was shown to be only 2.18 μg/L compared with 6.8 μg/L using the gold standard method; the % recovery of the urine assay by the frying pot method was as high as 98.2%. For water, the MDC was as low as 0.77 μg/L, (n=20) and the % recovery was 94.8.

Table 2 showed the intra- and inter-assay of frying pot using paraffin oil and water at 100°C.
TABLE 4a. Measurements in oil and water in 255 cases with a categorization of urine iodine deficiency, normal and excessive.

<table>
<thead>
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<th>Gold Standard</th>
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<tbody>
<tr>
<td></td>
<td>Iodine Deficiency</td>
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<tr>
<td></td>
<td>(n=63)</td>
</tr>
<tr>
<td>Frying pot using oil</td>
<td></td>
</tr>
<tr>
<td>Iodine Deficiency</td>
<td>50(79.4%)</td>
</tr>
<tr>
<td>Iodine Normal, Excessive</td>
<td>13(20.6%)</td>
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<tr>
<td>Frying pot using water</td>
<td></td>
</tr>
<tr>
<td>Iodine Deficiency</td>
<td>59(93.6%)</td>
</tr>
<tr>
<td>Iodine Normal, Excessive</td>
<td>4(6.3%)</td>
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The results shown in Tables 1 and 2 demonstrate that the frying pot method using water digestion is reproducible, sensitive and accurate when compared with the frying pot method using paraffin oil.

Similarities of standard curves of the two methods:

Fig 3 a, b and c displayed a comparison of standard curves between two frying pot methods and gold standard method. For two frying pot methods (using oil or water), standard iodine concentrations (0, 20, 40, 80, 120, 160, 200 μg/L) were plotted against the time of color change from blue to violet (Fig 3a and 3b). For the gold standard method (Fig 3c) iodine concentrations were plotted against the fading reaction of reagents as different optical densities. The three standard curves showed a very similar pattern.

Correlation between values for urine iodine samples by the frying pot and gold standard (heating block and spectrophotometer) methods as shown below

Fig 4x and 4y show urine iodine measurements from the frying pot using oil and the gold standard method, and the frying pot using water and the gold standard method, having a high positive correlation (r<sub> </sup> in oil = 0.914, n= 1,007 and r<sub> </sup> in water = 0.939, n= 1,007, respectively as shown in Table 3).

TABLE 4b. Showing the results of a bigger series into 1,007 cases using paraffin oil and water for digestion.

<table>
<thead>
<tr>
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<th>Gold Standard</th>
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<tbody>
<tr>
<td></td>
<td>Iodine Deficiency</td>
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<tr>
<td></td>
<td>(n=408)</td>
</tr>
<tr>
<td>Frying pot using oil</td>
<td></td>
</tr>
<tr>
<td>Iodine deficiency</td>
<td>377(92.4%)</td>
</tr>
<tr>
<td>Iodine normal, excessive</td>
<td>31(7.6%)</td>
</tr>
<tr>
<td>Frying pot using water</td>
<td></td>
</tr>
<tr>
<td>Iodine deficiency</td>
<td>370(90.7%)</td>
</tr>
<tr>
<td>Iodine normal, excessive</td>
<td>38(9.3%)</td>
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</table>

The prevalence of iodine deficiency in this study was 24.7% (63/255) which was similar to the prevalence in the Thai rural population. Therefore, all predictive values obtained from this study could be directly applied to the Thai rural population.

Table 4b demonstrated sensitivity and specificity of frying pot using oil and water in another series of 1,007 subjects. These sensitivity and specificity were in a good agreement with those from Table 4a. Since the prevalence of iodine deficiency in Table 4b was much higher (40.5%) than the general Thai rural population, predictive values were not presented.

It should be mentioned that using water for digestion in the frying pot provided the best sensitivity, specificity and accuracy.

**DISCUSSION**

The degree of iodine deficiency is demonstrated by the following iodine values. Iodine deficiency is generally associated with low urinary excretion rates of less than 20, 50 to 100 μg/L of iodine per 24 hours approximately.<sup>5,12</sup> The results of urine iodine can be categorized into four deficient groups, i.e., severe (<20 g/L), moderate (20-50 g/L), mild (50-70 g/L) and equivocal (70-100 g/L). A low iodine value is associated with more severe complications, e.g., mental and physical deficiency. Normal values (100-200 g/L) and excess (>200 g/L) are in the iodine replete groups.

Many advantages are derived from this frying pot method, particularly accuracy. The frying pot for the field method can avoid the high cost of heating blocks, perchloric fume hood and spectrophotometer. The SK reagents, prepared after Robbins,7 gave a change of color that can be easily differentiated. The time of color change from blue to violet in par-
affin oil and later in water in the frying pot method was accurately determined, indicating the exact quantitative concentration of urine iodine (Fig 3a and 3b). This accuracy was reconfirmed by the large series (n = 1,007). Urine samples, no matter how long they are kept in a freezer, can be digested by ammonium persulfate and give similar accuracy.

This study has shown that this new frying pot method provides good sensitivity and specificity. This new method is convenient for field use because of its portability. It simply requires water for digestion and needs no conventional fume hood. The bath water technique of the frying pot method can be readily carried out anywhere outside a laboratory by personnel with minimal training. By using only a stop-watch, construction of the standard curve for iodine concentrations in urine is simple and the time for color change from blue to violet can easily be observed. Moreover, the frying pot with the water method is safe and rapid because of non-explosive ammonium persulfate and needs only 25 minutes at 100°C instead of one hour for perchloric acid. In the field, using three frying pots and adequate numbers of laboratory personnel, the maximum number of samples tested per day can rise to 240.

Testing by the use of water in the frying pot can reduce the cost from 30 Baht 11 to <5 Baht per person (about US $ 0.10), at the current exchange rates. This makes the procedure affordable for project managers, villager and schools. Therefore, it is a satisfactory field method integrated into the list of techniques of measurement of iodine for evaluating and monitoring the IDD project in recent publications.4-16

CONCLUSION

This new modified field method of iodine detection in urine is simple, cheap and portable, using frying pot with water for digestion for rapid and reliable results. It is satisfactory for quantitative measurements of urinary iodine concentrations in a large number of urine samples in a day. By replacing paraffin oil the method uses water for digestion and improves the monitoring. The mathematic measurement can be very practical in monitoring and evaluating epidemiological studies in developing countries.

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REFERENCES