สารศิริราช
SIRIRAJ HOSPITAL GAZETTE

จัดพิมพ์โดยอนุมัติคณะกรรมการคณะแพทยศาสตร์ศิริราชพยาบาล
Published Under the Auspices of the Faculty of Medicine, Siriraj Hospital

ปีที่ 53, ฉบับที่ 10, ตุลาคม 2544	Volume 53, Number 10, October 2001

Rapid Detection of Iodine in Iodised Salt and Iodised Water Essential in a Program of Iodine Deficiency Disorders Control

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Abstract : **To determine and develop rapid on-site testing of iodine in salt and water samples and its use as field methods for monitoring and evaluation of the Iodine Deficiency Disorders (IDD) program. Materials and methods: In order to monitor iodine in salt, the single bottle technique is recommended, utilizing SHMP (May & Baker), tapioca flour, KI and sodium azide. To monitor iodine in water, the double bottle technique is employed and this utilizes 3N HCl as a substitute for SHMP to acidify flour, KI and sodium azide.**

Results : **In salt, it is not positive until the colour starts to change from brown to black. In water, when it is positive reaching beyond an optimum level at 100-200 µg of iodine per litre, the colour starts to appear as faint blue and becomes darker blue as concentration of iodine increases.**

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Discussion: Statistically, Spearman rank correlation co-efficient and the prediction method showed approximation of the two methods in estimation of iodine levels, indicating the interchangeability of the eyesight, and it could be used in screening in place of the spectrophotometer.
Conclusion: These techniques are cheap, simple and effective. This allows the monitoring kit to be beneficial and easy to use. These methods are "ordinary" and "natural", and thus, in harmony with Thai culture and customs.

เรื่องย่อ : **การตรวจไอโอดีนอย่างเร็วในเกลือเสริมไอโอดีนและน้ำเสริมไอโอดีนที่จำเป็นสำหรับโปรแกรมการควบคุมโรคขาดสารไอโอดีน**

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สารศิริราช 2544; 53: 721-728.

การศึกษานี้มีจุดประสงค์เพื่อพัฒนาวิธีตรวจไอโอดีนในเกลือและน้ำเสริมไอโอดีน ชนิดใช้ในสนามอย่างรวดเร็ว เพื่อใช้ในการตรวจและประเมินผลของโรคขาดสารไอโอดีน

ในการตรวจไอโอดีนใน*เกลือเสริมไอโอดีน*ใช้เทคนิค*ขวดเดี่ยว* บรรจุโซเดียมเฮกซาเมตาฟอสเฟต (เอสเอชเอ็มพี ของบริษัท เมย์ แอนด์ เบเกอร์) แป้งมัน และโปแตสเซียมไอโอไดด์ โดยมีโซเดียมเอไซด์เป็นสารกันบูด เมื่อหยดสารละลายนี้ลงในเกลือ ในรายที่ให้ผลบวกจะเป็นสีน้ำตาลและเริ่มเปลี่ยนเป็นสีดำ เกลือเสริมไอโอดีนที่พอเหมาะในการบริโภคของประชากรที่ขาดสารไอโอดีนจะมีสีน้ำตาลและกลายเป็นสีดำเข้มขึ้นมากและน้อยตามจำนวนของไอโอดีนที่เสริมในเกลือ วิธีนี้สะดวกต่อการพกพา และง่ายต่อการใช้

ในการตรวจไอโอดีนใน*น้ำเสริมไอโอดีน* ใช้เทคนิคขวดคู่ ซึ่งเหมือนกับวิธีแรกทุกประการ เว้นแต่มีกรดไฮโดรคลอริก 3 นอร์มัล แทน เอสเอชเอ็มพี อยู่ในอีกขวดหนึ่ง เมื่อหยดลงในน้ำ ถ้ามีไอโอดีนน้ำนั้นจะเริ่มเกิดสีฟ้าอ่อน แสดงว่ามีขนาดของไอโอดีนพอเหมาะ ถ้าเป็นสีฟ้าแก่มากขึ้นหรือเป็นสีน้ำเงิน แสดงว่ามีไอโอดีนมากกว่าปกติ

เทคนิคการใช้ตาดูการเปลี่ยนแปลงของสี เหมาะสำหรับการตรวจสอบเกลือและน้ำในภาคสนาม เทคนิคการตรวจทั้งสองอย่างนี้มีราคาถูก ทำได้ง่าย และได้ผลดี กระบวนการนี้เป็นไปในลักษณะที่ผสมผสานกับธรรมชาติและกลมกลืนกับประเพณีและวัฒนธรรมของไทย

INTRODUCTION

Frequent monitoring of iodine levels in salt and water[1,2] is essential for a successful iodine control program to check that dietary iodine is adequate. If it is inadequate, then appropriate correction is necessary.

In Thailand, a team at Siriraj Hospital distributes iodine as KIO_3 in both salt and water. The concentration of iodine is 100 µg in 5 gm of salt, and 100 µg in 1,000 ml of water.[1] Such doses of iodine are made available through the Government daily to school children and villagers.

To monitor iodine levels, field kits for rapid on-site testing in salt and water have been developed. The objective of this project is to find methods of iodine detection, which are rapid, reliable and can be used "on site" in the field. The techniques show iodine levels semi-quantitatively in both salt and water samples. The essentials are performed by adding to salt one drop of detecting solution of the single bottle, which begins to change from brown to black in a positive sample. For water, another bottle of hydrochloric acid (replacing SHMP) is added, and the double bottles detecting kit formed, producing, blue colour[3] in positive cases.

MATERIALS AND METHODS

Rapid detection of iodine in salt (by the single bottle technique).[4,5]

A 300 ml bottle contains the following reagents: 1.5 gm of tapioca flour (1%) boiled in 150 ml of hot water and left to cool, 4.5 gm of KI (3%) and 0.75 gm of sodium azide (0.5%) are added to the solution as preservatives. The solution is mixed with 150 ml of 1% sodium hexameta-phosphate (SHMP) with a pH of approximately 6.0. The SHMP is obtained from May& Baker.

Measurement: A drop of the indicator from the single bottle is added to small amount of iodised salt. A faint brownish colour develops immediately and becomes brown/black within one minute in sample of 30-50 parts per million (ppm) iodine. When positive, the salt becomes black. The rate of production and intensity of the colour indicate the level of iodine in any sample. SHMP is used to acidify the detecting solution, producing weak acid pH. Semi-quantitative analysis is shown using the colour scale on the label on the side of the bottle.

Rapid detection of iodine in water (by double bottles technique).

One drop of the first bottle (with no SHMP), containing the same concentration of KI, tapioca flour and sodium azide as iodine detecting solution in salt is added to 2 ml of iodised water sample. The solution is then acidified by another drop of 3N HCl from the second bottle, resulting in a minute amount of free iodine and a faint blue colour on contact with the flour. The rate of production and intensity of the colour increase with rising iodine concentration in water by 30 minutes.

The measurement of iodine in both salt and water were subjected to both methods of the single and double bottles and compared with those obtained by spectrophotometer. The statistics were treated by Statistical Package for Social Science (SPSS) program to confirm the accountability of the methods using eyesight against spectrophotometer.

RESULTS

Rapid detection of iodine in salt (by the single bottle technique).

Iodine in salt was detected rapidly by a single bottle technique.

Semi-quantitative detection of iodine in salt, the colour developed after addition of one drop of the single bottle solution to the salt (Figure 1).

It is noted that 30 ppm is the value of the minimum permissible iodine level in salt in Thailand.[6] The colour changes from brown to black at this concentration. The solution is stable for over 2 years. Each 30 ml bottle costs around 9.00 baht or US $ 0.21.

Rapid detection of iodine in water (by double bottle technique)

One drop of each solution from the first and second bottle was added to a 2 ml water sample. A blue colour developed and its intensity increased as

Colour scale of iodised salt

No colour	Brownish	Brown	Blackish	Black
Control	*10 ppm*	20 ppm	*Std. 30 ppm*	*50 ppm*

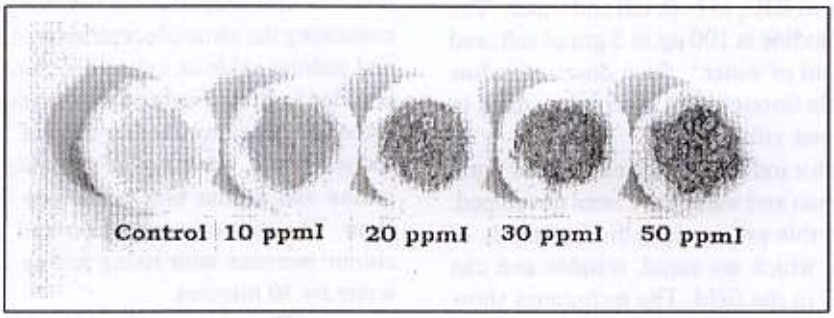


Figure 1. Showing colours of varying concentrations of iodine in the salt. The normal range is blackish to black colour.

Colour scale of iodised water

No colour	Very weak blue	Faint blue	Blue	Dark blue
Control	*Std. 100 µg /L*	*Std. 200 µg /L*	*300 µg /L*	*400 µg/L*

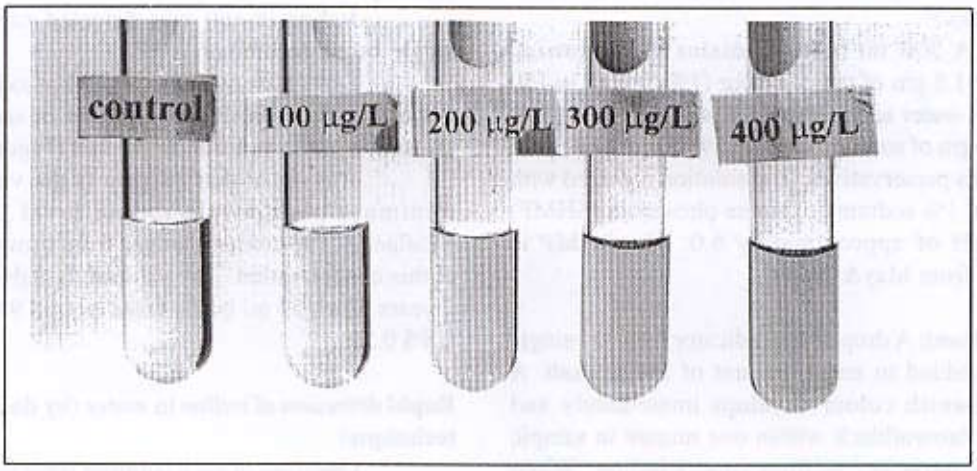


Figure 2. Showing changes in colour at 30 minutes in water containing various concentrations of iodine. The water sample begins to have faint blue colour when positive.

the level of iodine rised through normal, positive to excess.

Note the colour of iodised water at 100-200 µg/L which represent the concentration of iodine normally provided to children and villagers (Figure 2).

It is recommended that each person, child or adult, ingests 5 gm (or 100 µg of iodine) iodised salt and/or drink approximately 1,000 ml of iodised water (or 100-200 µg of iodine[7]) per day in order to supply the iodine needed for daily nutrition.

The double bottles kit of 30 ml remains stable for more than 2 years and costs approximately 7.00 baht or US $ 0.16.

Evaluation of indicators used in field and spectrophotometer methods

A. *Assay performance in the laboratory*

Results comparing between the two readings from salt and water, i.e. by eyesight and spectrophotometer, are presented in the previous article[8] and the following Table 1, Table 2 and Figure 3.

Table 1. Iodine levels in iodised salt as seen by eyesight and spectrophotometer.

Iodine levels in salt by eyesight (ppm) (x)	Iodine levels in salt by spectrophotometer (ppm) (y)				Spearman rank correlation coefficient (r)
	N	Mean	S.D.	Range	
0	18	2.71	1.97	0.28 - 6.67	
10	29	11.44	2.34	8.70 - 16.89	
20	9	20.31	2.09	16.76 - 22.35	0.954 ($p < 0.01$)
30	8	29.08	5.30	22.30 - 38.20	
50	38	48.00	12.81	36.32 - 65.30	$y = 2.38 + 0.91x$

Table 2. Iodine levels in iodised water as seen by eyesight and spectrophotometer.

Iodine levels in water by eyesight (µg/L) (x)	Iodine levels in water by spectrophotometer (µg/L) (y)				Spearman rank correlation coefficient (r)
	N	Mean	S.D.	Range	
0	17	46.4	31.1	2.8 - 94.5	
100	24	132.7	26.7	100.0 - 197.0	
200	11	242.7	21.7	213.6 - 280.6	0.968 ($p < 0.01$)
300	9	333.5	20.9	297.2 - 363.2	
400	22	405.5	29.6	370.8 - 486.1	$y = 48.53 + 0.89x$

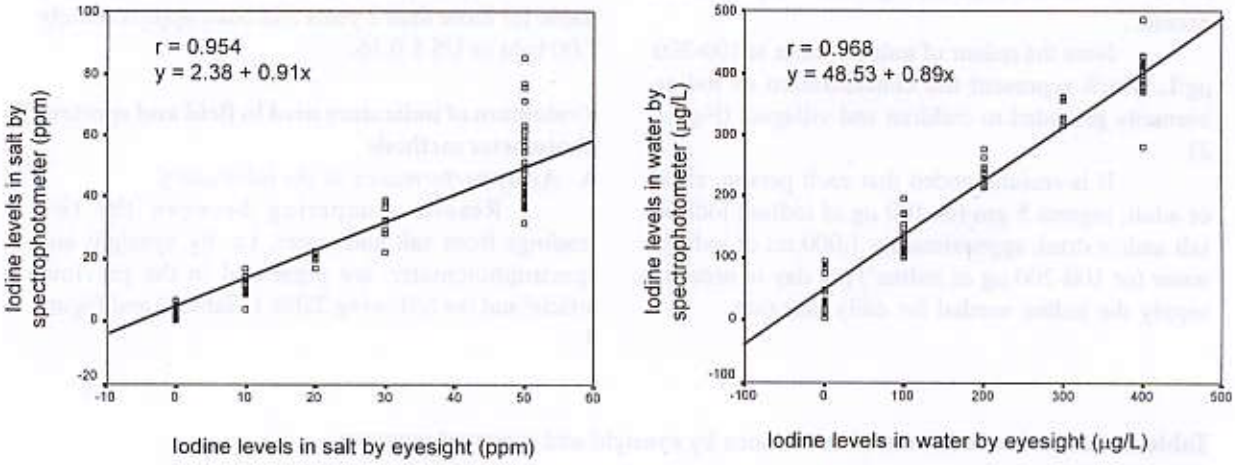


Figure 3. Showing iodine levels in salt and water by eyesight (x) and spectrophotometer (y).

The Spearman rank correlation coefficient showed very good positive association between iodine level by eyesight (x) and that by spectrophotometer (y) for salt ($r = 0.954$, $p < 0.01$, with regression equation of $y = 2.38 + 0.91x$), and for water ($r = 0.968$, $p < 0.0l$, $y = 48.53 + 0.89x$). These two simple linear regression equations can then be used to predict iodine level by spectrophotometer from that by eyesight. For example, with the observed iodine level in salt by eyesight of 20 and 40 ppm, the predicted iodine level by spectrophotometer will be 20.58 [$=2.38 + 0.91(20)$] and 38.78[$=2.38 + 0.91(40)$] ppm, respectively. These indicated the credibility of the methods used in public health for screening and evaluation of the IDD program.

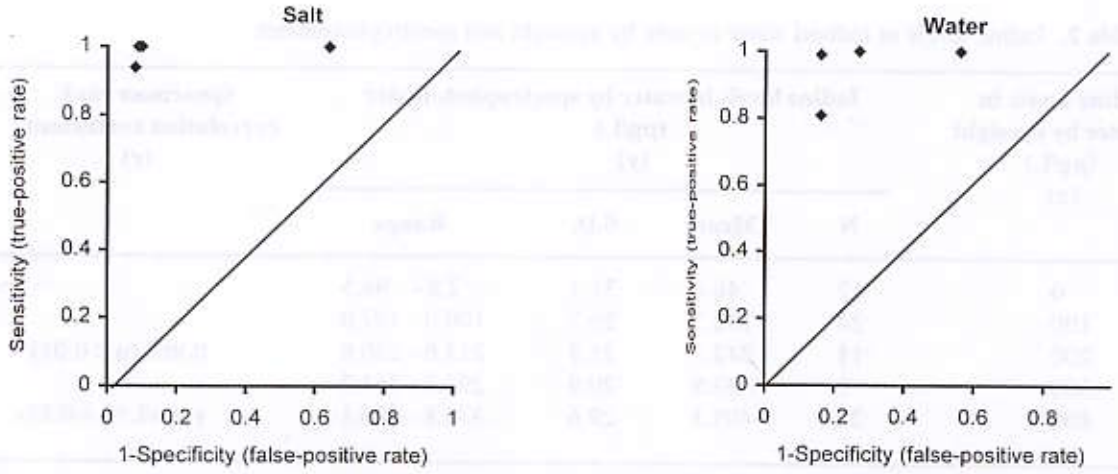


Figure 4. ROC curves in iodised salt and iodised water.

B. *Receiver Operating Characteristic (ROC) curves for iodine levels in salt and water* (Figure 4)

It revealed that the best cut point in salt was 30 ppm (sensitivity = 75/80 = 93.8%, 95% CI = 86.0 - 97.9 and specificity = 57/62 = 91.9%, 95% CI = 82.2 - 97.3), and that in water was 200µg/L (sensitivity = 81/81= 100%, 95% CI = 95.5 - 100, and specificity = 30/41= 73.2%, 95% CI = 57.1 - 85.8, respectively). Accuracy of the method for salt was 132/142 = 93.0% (95% CI = 87.4 - 96.6) while that of water was 111/122 = 91.0% (95% CI = 84.4 - 95.4).[9]

Five and 11 false positive cases were found in iodised salt and iodised water in the series of patients respectively. False negative was found in 5 and 0 cases for iodised salt and iodised water respectively.

DISCUSSION

Levels of iodine in iodised salt and iodised water

The Thai Government recommends a minimum concentration of KIO_3 in salt of 30 ppm per day,[6,10,11] while the daily physiologic intake should be 50 ppm or more. When measured in salt, the change of colour of the potassium iodate is from brown to black in a normal sample. In water, a level of 100-200 µg/L at which the colour changes to faint blue is recommended as a normal level. There is a wide margin of normal values for water due to much increased volume.

The non-uniform concentration of iodine in salt in the false positive and false negative originates from high temperature and humidity, causing the particles to adhere and form aggregations. As clarified by WHO, the inevitably iodine loss from salt between production and consumption is often much higher in non-industrialized countries, where packaging, storage and transportation conditions are poor. Expected iodine contents of salt should be established at different points along the distribution system, taking into account climatic conditions, types of packaging, and habitual daily salt consumption. Quality control is the responsibility of the Ministry of Public Health through primary health care unit and regional or provincial and district health divisions. The monitoring should be updated periodically, e.g. every two years, to ensure that iodine concentration levels are adequate, and if necessary, corrective action is taken.

In water, especially tapped water, the false positive error by early appearance of faint blue is from the presence of halides, i.e. chloride, bromide and fluoride, which are the natural contamination. Otherwise it is from the working ability of operator in miscalculating the iodine concentration of the prepared fresh iodised water.

By linking the two previous descriptions, A and B, it is concluded that assay performance of iodised salt and water by eyesight can be used in screening of the samples as that by spectrophotometer.

Checking iodised salt should be performed twice a year, using the described technique of the single bottle which has the advantages of being simple to use with only one bottle containing SHMP, and immediate results obtained in minutes, and, can be readily distributed and available to villagers. The chemical reaction is that a weak acid solution of SHMP liberates iodine on contact with flour, which turns a faint brown to black colour. Occasionally the samples of salt should be checked in the laboratory for repeat iodisation with a single bottle of solution containing SHMP, and/or by spectrophotometer for accuracy and certainty.

For iodised water, a school child or villager should drink about 1, 000 ml of 100-200 µg of iodine[12] as potassium iodate daily; and in early positive case, appearance of blue colour develops in the water may be detected by a double bottles method. Samples that do not exhibit any change in colour are negative. In Thailand, potassium iodate is provided free of charge to villagers and school children, and the iodised water is given during daily lunch in school.

Iodised salt and iodised water should not provide more than 200 µg of iodine per person per day, which is the dose that still keeps TSH within normal limits.[13] At this dosage of iodine, thyroid function should return to normal and goitres disappear in one or more years. There is, however, an evidence that very large dose (1,500 µg) of iodine daily for 78 days produces iodine induced hyperthyroidism or Jod-Basedow phenomenon.

However, Bourdoux et al, reported that after 2 years of iodine prophylaxis with iodised salt (approximately 100 ppm or more) in Zaire, urinary excretion of 200 to 500 µg daily were resulted. Fourteen per cent of 190 subjects had undetectable TSH, increased T4 and T3, and symptoms of iodine induced hyperthy-roidism, requiring anti-thyroid drug treatment. The induced hyperthyroidism in occasion occurs in the elderly, with/without underlying diseases, nodular/diffuse goitre, with/without autonomous nodule, in endemic iodine deficient area. Daily iodine intakes of up to l mg, i.e. 1,000 µg, and 150-300 µg/day appear to be entirely safe. Daily consumption of 10 gm of iodised salt containing 50 PPM would add a maximum of only 500 µg of iodine and thus the likelihood of exceeding an iodine intake of 1 mg/day from iodised salt is quite small. It can be seen that even at this dosage, wide margins are always provided before development of this disorder.[14-16]

The benefits of iodine supplementation far outweigh any risk of excess iodine intake for a small minority. The cost of the salt and water is rather small. School teachers take responsibility for the financial management and administration of both iodised salt and iodised water. In a properly run program, the villagers willingly provide financial support to help keep their children healthy.

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