

Validation of spot-testing kits to determine iodine content in salt

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Iodine deficiency disorders are a major public health problem, and salt iodization is the most widely practised intervention for their elimination. For the intervention to be successful and sustainable, it is vital to monitor the iodine content of salt regularly. Iodometric titration, the traditional method for measuring iodine content, has problems related to accessibility and cost. The newer spot-testing kits are inexpensive, require minimal training, and provide immediate results. Using data from surveys to assess the availability of iodized salt in two states in India, Madhya Pradesh and the National Capital Territory of Delhi, we tested the suitability of such a kit in field situations. Salt samples from Delhi were collected from 30 schools, chosen using the Expanded Programme on Immunization (EPI) cluster sampling technique. A single observer made the measurement for iodine content using the kit. Salt samples from Madhya Pradesh were from 30 rural and 30 urban clusters, identified by using census data and the EPI cluster sampling technique. In each cluster, salt samples were collected from 10 randomly selected households and all retailers. The 15 investigators performing the survey estimated the iodine content of salt samples in the field using the kit. All the samples were brought to the central laboratory in Delhi, where iodine content was estimated using iodometric titration as a reference method. The agreement between the kit and titration values decreased as the number of observers increased. Although sensitivity was not much affected by the increase in the number of observers (93.3% for a single observer and 93.9% for multiple observers), specificity decreased sharply (90.4% for a single observer and 40.4% for multiple observers). Due to the low specificity and resulting high numbers of false-positives for the kit when used by multiple observers ("real-life situations"), kits were likely to consistently overestimate the availability of iodized salt. This overestimation could result in complacency. Therefore, we conclude that until a valid alternative is available, the titration method should be used for monitoring the iodine content of salt at all levels, from producer to consumer, to ensure effectiveness of the programme.

Keywords: sodium chloride, analysis; iodine content; colorimetry, methods; evaluation studies; India.

Voir page 979 le résumé en français. En la página 979 figura un resumen en español.

Introduction

Universal salt iodization (USI) is the most widely practised intervention in eliminating iodine deficiency disorders (IDDs) (1). Salt iodine testing is an important "process" indicator for monitoring progress towards USI. Under the National Iodine Deficiency Disorders Control Programme in India, iodization of salt is the recommended strategy, with the level of iodization fixed at a minimum of 15 parts per million (ppm) at the consumer level and 30 ppm at the production level. Most Indian states have introduced mandatory salt iodization through legislation (2). The salt department and the state governments are responsible for monitoring the salt iodine content at both the production and consumption levels (2).

Iodometric titration, the traditional method for determining iodine content, is an accurate method (3). But it is time-consuming, and requires capital infrastructure and trained personnel. In India, such laboratories are currently located either at the district or state headquarters. Hence, the length of time between collecting the sample and the availability of results is considerable.

Iodine spot-testing kits do not require any infrastructure, are inexpensive, and most importantly, provide immediate results suitable for rapid feedback. The kits give either a qualitative or a semiquantitative estimation of the iodine content. "Qualitative" means that salt samples are classified as (adequately) iodized or uniodized. The test gives no indication of the actual level of iodine in the salt. Some kits give a semiquantitative estimate of iodine content of the salt. For example, one kit categorizes salt samples as having an iodine content of 0, 7, 15, or 30 ppm. In addition to monitoring, iodine spot-testing kits are powerful tools for advocating good health and educating consumers about health. Programme managers in several countries have been quick to adopt the use of kits, and occasionally use

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them as the sole tool for monitoring iodine content (4).

Previous studies of the validation of iodine spot-test kits for the analysis of salt have been largely laboratory-based or used a single observer (5–7). However, there is a need to test the kits in a field situation where multiple observers are involved in their use. In this study we address the validation and agreement of the iodine spot-testing kit used in actual field conditions in two Indian states, as compared with the iodometric titration method.

Methods

The data for the present study were collected during two USI surveys carried out by our team in the state of Madhya Pradesh (central India) in 1995 and the National Capital Territory of Delhi (northern India) in 1996.

The survey in Madhya Pradesh was a part of the independent survey evaluation of USI carried out in November 1995 (8). The EPI 30 cluster sampling method based on probability proportional to size of cluster was followed (7). The state was divided into rural and urban strata. In each stratum, 300 households were selected, i.e. 10 in each cluster. The study team comprised 15 senior physicians from different national institutions and medical colleges in India. In each cluster, salt samples were collected from selected households and all the retail shops in that village or urban cluster. The samples were tested in the field by physicians using the salt-testing kit.

The survey in the National Capital Territory of Delhi was a school-based survey carried out to estimate the use of iodized salt at the household level (9). Thirty schools from South Delhi were selected using the EPI 30 cluster sampling methodology. After briefing the school principals, teachers, and students about IDD and the importance of consuming adequately iodized salt at home, the children from grades 6 to 8 were requested to bring salt samples from their homes on a prearranged day. On that day, a public health nurse from the project visited the school and tested the salt samples using the iodine spot-testing kit. The results were communicated to the students and teachers, and the opportunity was also used to reinforce messages about IDD. In both surveys, the investigators were given training for half a day in using the spot-testing kit and collecting salt samples for transportation to Delhi.

About 20 g of salt samples were collected from each selected household in airtight plastic envelopes and brought to the Iodine Monitoring Laboratory of the International Council for Control of Iodine Deficiency Disorders (ICCIDD) located at the Centre for Community Medicine, All India Institute of Medical Sciences, New Delhi, for iodometric titration.

Laboratory procedures

The test kit, produced by MBI chemicals, Chennai (India), is a starch-based test. According to the

manufacturer's instructions, the test can be used semiquantitatively to measure iodine in salt at 0, 7, 15, and >30 ppm, depending on the intensity of the colour obtained. If the test showed "no iodine" on the first testing, the test solution was added again after acidifying the salt sample. This was required to neutralize the presence of alkali in the salt. If the test showed no iodine when tested for a second time, this was taken as the true test result. The kits were used at least eighteen months before the expiry date marked on the pack.

Iodometric titration was the reference method used to estimate the iodine content of salt. This method uses the thiosulfate–starch reaction as an external indicator (10). Salt samples were analysed for their iodine content by carrying out single titrations using 10 g of salt. All the samples were tested within 1 month of collection. Analysis was carried out under the supervision of a senior biochemist (M.G.K.). For internal quality control, one iodized salt sample was tested 20 times for standardization. For every 75 test samples, 1 standard sample was retested. The value for the standard was within the acceptable limits.

After the two surveys had been completed, as part of our routine procedure to assess agreement, two laboratory workers were asked to test 100 consecutive salt samples sent to the laboratory using spot-testing kits identical to those used in the survey. The two workers chosen had been working for about 2 years in our laboratory.

Data analysis

The data were processed and analysed using Epi Info 6 (11). Tests for proportion and 95% confidence intervals were estimated as appropriate. To validate the spot-testing kit, sensitivity and specificity were calculated. The agreement was assessed using the κ statistic.

The main purpose of the study was to assess the performance of the kit in monitoring the iodine content of salt in situations of multiple observers. The goal of monitoring USI is to estimate the availability of "adequately" iodized salt (≥ 15 ppm of iodine). The results were presented accordingly. In addition, results were also divided into two groups and analysed according to the presence (>0 ppm) or absence (0 ppm) of iodine in the salt.

In order to compare the performance of the kit with iodometric titration, distribution curves of the iodine content of the salt sample as estimated by titration were plotted separately for categories labelled as 0, 7, 15, and 30 ppm by the kit.

Results

The results of the study are presented separately for single and multiple observers.

Single-observer data from Delhi

A total of 1258 salt samples were collected from schools in Delhi and tested both by titration and by

kit. Both methods showed a similar proportion of salt samples with iodine ≥ 15 ppm (63.6% by kit and 64.4% by titration) (Table 1). However, most of the samples found by kit to have 0 ppm did have some iodine in them. The sensitivity (ability to correctly identify salts with adequate iodine content) of the kit was 93.3% (95% confidence interval (CI) = 91.3–94.9) and specificity (ability to correctly identify salts with inadequate iodine content) was 90.4% (95% CI = 87.2–92.9). The positive predictive value was 88.2% (95% CI = 84.8–90.9) while the negative predictive value was 94.6% (95% CI = 92.8–96.0) (Table 2).

Multiple-observer data from Madhya Pradesh

A total of 682 salt samples was collected from retail shops and households in Madhya Pradesh. The kit overestimated iodine content as compared with titration owing to a high number of false-positives. The proportion of samples classified as having an iodine level >15 ppm was 83.4% using the kit as compared with 69.5% using the titration method (Table 3). As observed in Delhi, most of the samples that tested as 0 ppm using the kit contained some iodine. With multiple observers, the spot-testing kit reporting adequate and inadequate iodine had high sensitivity (93.9%) (95% CI = 91.2–95.8) but poor specificity (40.4%) (95% CI = 33.7–47.4) in detecting adequately iodized salt. The positive predictive value was 78.2% (95% CI = 74.5–81.5%) while the negative predictive value was 74.3% (95% CI = 65.1–81.9) (Table 2).

Salt samples were also divided into two groups: iodine present or iodine absent. The results were similar to those described above with specificity and agreement decreasing for multiple observers as compared to a single observer. However, there were very few samples in the not iodized category.

We plotted single-observer data by iodine content as assessed by the kit against titration results

(Fig. 1). A large overlap was observed for the actual iodine content of the salt sample for the values of 0, 7, and 15 ppm as assessed by the kit. The salt samples assessed at 30 ppm by the kit showed a clear demarcation from the rest. Thus, even with a single observer, the kit failed to discriminate between iodine contents of 0, 7, and 15 ppm.

In our laboratory, interobserver variation between two people with 2 years' experience using the kit was found to be acceptable ($\kappa = 0.64$). However, the agreement for samples with 7 or 15 ppm was low ($\kappa = 0.34$), which confirmed the overlap mentioned above.

Discussion

This study analyses the performance of spot-testing kits in evaluating the status of salt iodization in two states of India using single and multiple observers. There are two limitations of the study which must be kept in mind. First, the data used for analysis were not originally collected for this comparison. This limitation does however eliminate any bias due to the

Table 1. Comparison of spot-testing kits versus the iodometric titration method for the determination of iodine content in salt: single-observer data^a

Spot-testing kit (ppm of iodine)	Iodine concentration by iodometric titration (ppm)			Total
	0	0.1–14.9	≥ 15	
0	6	287	27	320 (25.4)
7	0	111	27	138 (11.0)
15 and 30	0	43	757	800 (63.6)
Total	6 (0.5)^b	441 (35.1)	811 (64.4)	1258

^a Data from Delhi.

^b Values in parentheses are percentages.

Table 2. Validation of the iodine spot-testing kit as a qualitative method

Interpretation of test	Indicator (%)			
	Sensitivity ^a	Specificity ^a	PPV ^b	NPV ^c
Iodine present (> 0 ppm) versus iodine absent (0 ppm)				
Multiple-observer data ^d	93 (90.0–95.0)	14.3 (0.3–57.8)	99.0 (98.0–99.6)	2.0 (0.1–12.0)
Single-observer data ^e	74.9 (72.4–77.3)	100.0 (54.0–100.0)	100 (99.5–100.0)	2.0 (0.8–4.2)
Iodine adequate (≥ 15 ppm) versus iodine inadequate (< 15 ppm)				
Multiple-observer data ^d	93.9 (91.2–95.8)	40.4 (33.7–47.4)	78.2 (74.5–81.5)	74.3 (65.1–81.9)
Single-observer data ^e	93.3 (91.3–94.9)	90.4 (87.2–92.9)	88.2 (84.8–90.9)	94.6 (92.8–96.0)

^a Values in parentheses are 95% confidence intervals.

^b PPV = positive predictive value.

^c NPV = negative predictive value.

^d Data from Madhya Pradesh.

^e Data from Delhi.

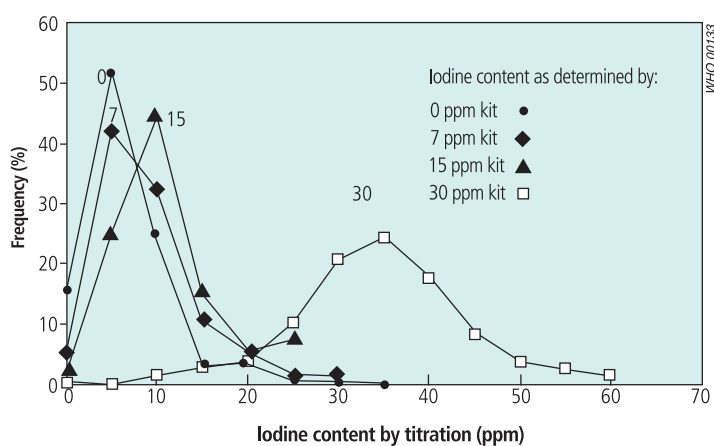
Table 3. Comparison of spot-testing kits versus the iodometric titration method for the determination of iodine content in salt: multiple-observer data^a

Spot-testing kit (ppm of iodine)	Iodine concentration by iodometric titration (ppm)			Total
	0	0.1–14.9	≥ 15	
0	1	38	9	48 (7.1)
7	2	43	20	65 (9.5)
15 and 30	4	120	445	569 (83.4)
Total	7 (1.0)^b	201 (29.5)	474 (69.5)	682

^a Data from Madhya Pradesh.

^b Values in parentheses are percentages.

Fig. 1. Spot-testing kits versus the iodometric titration method for the determination of iodine content in salt



Data from Delhi only ($n = 1258$)

investigators having prior knowledge of the hypothesis. Second, very few samples in the study area contained no iodine. This is to be expected where salt iodization has already been introduced.

The study showed that the use of multiple observers resulted in a sharp decline in agreement between the titration method and the kit when iodine content was used either as a qualitative (adequate/inadequate) or as a semiquantitative tool (four levels: 0, 7, 15, and 30 ppm). The sensitivity of the kit in differentiating adequate and inadequate iodine content of salt was similar for single and multiple observers. However, the number of false-positives was much higher with multiple observers. Multiple observers simulate actual field conditions. Some of the difficulties that arise in using the kit are due to the fact that the kit manufacturers do not precisely make clear what a positive test result of, for example, 15 ppm means. Greater than 15 ppm, approximately 15 ppm, 11–22.5 ppm or 7–30 ppm all satisfy the criterion for a positive result. This also applies to the spot-test kits for other concentrations (0, 7, 30 ppm).

The variability in the results for the same kit was also shown in a multicentric study conducted in

India (7). Although the kit was used by a single observer at each centre, the results among the eight centres were quite variable. The sensitivity varied between 81% and 95.5%, but specificity varied from 50.4% to 100%.

In monitoring salt iodization, the objective is to ensure that the community consumes adequately iodized salt. False-positives would lead to overestimates of iodine content. This could result in complacency on the part of the programme managers. Incorrectly classifying a sample as “inadequate” when it actually has “adequate” iodine (false negative) would result in extra efforts and waste scarce resources. As evident from the current study, the spot-testing kit appeared to have similar sensitivity with both single and multiple observers. Hence, underestimating programme performance is less likely. Because the number of false-positives are indirectly proportional to prevalence, the error diminishes as the availability of adequately iodized salt improves. This phenomenon would result in underestimates of the progress made to make iodized salt available.

In Madhya Pradesh, before the current survey was carried out, programme managers claimed that the availability of adequately iodized salt was 84.21% according to their internal monitoring system. This figure was based on 80 000 salt samples in 42 districts collected and tested by over 4000 health workers. The availability of adequately iodized salt to the community was overestimated by >20%. Because of the high number of false-positives, programme performance was overrated and resulted in complacency among programme managers and field staff. Moreover, if inadequately validated tests are used for monitoring a public health programme, the community as well as the programme managers may lose faith in the effectiveness of intervention. Such an outcome could lead to decreases in the efforts to control IDD and the consequent persistence of IDDs in the community.

We have presented results of the performance of one particular kit that is used extensively in India. However, many other kits are available and are being used worldwide (12). Also, the recommended levels of iodine in salt vary from country to country. The performance of any particular kit at relevant levels of iodization needs to be assessed for each country separately. Our study emphasizes the need for a full evaluation of the spot-testing kit using multiple observers before introducing it to monitor programmes at the national level.

Iodine spot-testing kits for salt analysis are very useful components of public health programmes. Thus, efforts should be made to improve the kits that are currently available. Until a valid alternative is available, the titration method should continue to be used for monitoring the iodine content of salt collected from production sites, distribution points (retail shopkeepers), and households. ■

Résumé

Validation des nécessaires permettant de tester ponctuellement la teneur en iode du sel : expériences indiennes

Les troubles dus à une carence en iode constituent un problème important de santé publique et l'iodation du sel est l'intervention la plus largement employée pour les éliminer. Toutefois, il est indispensable de surveiller régulièrement la teneur en iode du sel pour que cette intervention soit efficace et durable. Le dosage iodométrique, méthode traditionnelle de mesure de la teneur en iode, présente des problèmes du fait qu'il est peu accessible et coûteux. Les nouveaux nécessaires d'épreuves utilisables pour des tests ponctuels sont peu coûteux, exigent une formation minimale et donnent immédiatement les résultats. À l'aide des données des enquêtes visant à évaluer la disponibilité en sel iodé dans deux États indiens, le Madhya Pradesh et le Territoire de Delhi, nous avons testé l'applicabilité d'un tel nécessaire sur le terrain. Des échantillons de sel ont été recueillis dans 30 écoles de Delhi, choisies en appliquant la technique du sondage en grappes du Programme élargi de Vaccination. Un seul observateur s'est chargé de mesurer la teneur en iode au moyen du nécessaire. Les échantillons de sel recueillis dans le Madhya Pradesh l'ont été dans 30 grappes rurales et 30 grappes urbaines identifiées par la même technique, en se servant des données du recensement. Dans chaque grappe, les échantillons de sel ont été recueillis dans 10 foyers choisis au hasard et chez tous les détaillants. Les

15 enquêteurs ayant procédé à cette enquête ont fait une estimation de la teneur en iode des échantillons de sel sur le terrain au moyen du nécessaire d'épreuve. Tous les échantillons ont été ramenés au laboratoire central de Delhi, où la teneur en iode a été estimée au moyen du dosage iodométrique, qui a servi de référence. La concordance entre les valeurs obtenues avec le nécessaire d'épreuve et le dosage iodométrique diminuait au fur et à mesure que le nombre d'observateurs augmentait. Si la sensibilité n'a pas été très touchée par l'augmentation du nombre d'observateurs (93,3 % pour un seul observateur et 93,9 % pour de nombreux observateurs), la spécificité a chuté brutalement (90,4 % pour un seul observateur et 40,4 % pour de nombreux observateurs). Étant donné la faible spécificité et le grand nombre de faux positifs qui en est résulté lorsque le nécessaire d'épreuve a été employé par de nombreux observateurs (« situation réelle »), il est probable que ces derniers surestiment régulièrement la disponibilité en sel iodé. Cette surestimation pourrait entraîner un relâchement des efforts. Par conséquent, jusqu'à ce qu'on dispose d'une autre méthode fiable, il faut utiliser le dosage iodométrique pour surveiller la teneur en iode du sel à tous les échelons, depuis le producteur jusqu'au consommateur, si l'on veut s'assurer de l'efficacité du programme.

Resumen

Validación de kits de determinación in situ del contenido de yodo de la sal: experiencia en la India

Los trastornos por carencia de yodo son un grave problema de salud pública, y la yodación de la sal es la intervención más empleada para eliminarlos. Sin embargo, es fundamental vigilar regularmente el contenido de yodo de la sal para asegurar el éxito y la sostenibilidad de esa intervención. El método empleado tradicionalmente para medir el contenido de yodo, la valoración yodométrica, plantea problemas relacionados con su accesibilidad y costo. Los kits más recientes para análisis in situ son baratos, apenas exigen adiestramiento, y permiten obtener resultados inmediatos. Partiendo de los datos aportados por estudios de evaluación de la disponibilidad de sal yodada en dos Estados de la India, Madhya Pradesh y el Territorio de Delhi, procedimos a analizar la utilidad del kit sobre el terreno. En Delhi se obtuvieron muestras de sal de 30 escuelas, empleando para ello la técnica de muestreo por conglomerados del Programa Ampliado de Inmunización (EPI). Las mediciones del contenido de yodo mediante el kit fueron efectuadas por un solo observador. Las muestras de sal de Madhya Pradesh procedían de 30 conglomerados rurales y 30 urbanos, identificados mediante la técnica de muestreo por conglomerados del EPI a partir de datos censales. En cada grupo, se obtuvieron muestras de sal a partir de 10 hogares seleccionados al azar y de todos los

minoristas. Los 15 investigadores participantes en el estudio calcularon el contenido de yodo de las muestras de sal empleando el kit sobre el terreno. Todas las muestras se llevaron al laboratorio central de Delhi, donde el contenido de yodo se determinó mediante la valoración yodométrica como método de referencia. El grado de coincidencia entre los niveles obtenidos con el kit y los obtenidos mediante la valoración disminuyó paralelamente al aumento del número de observadores. Aunque la sensibilidad no se vio demasiado afectada por el aumento del número de observadores (93,3% para un solo observador, y 93,9% para varios observadores), la especificidad disminuyó pronunciadamente (90,4% para un solo observador, y 40,4% para varios observadores). Considerando la baja especificidad y el número consiguientemente elevado de falsos positivos obtenidos cuando lo usaron varios observadores («condiciones reales»), cabe concluir que el kit tendía a sobreestimar sistemáticamente la disponibilidad de sal yodada. Puesto que esa sobreestimación podría conducir a la complacencia, mientras no se disponga de una alternativa válida debería seguirse utilizando el método de valoración para controlar el contenido de yodo de la sal en todos los niveles, desde el productor hasta el consumidor, a fin de garantizar la eficacia del programa.

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