

Research Article

Development and Validation of Rapid Test Kit for Qualitative Assessment of Iodine Content in Salts

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Email: toqeersaqi2853@gmail.com**Received:** November 01, 2024; **Accepted:** November 20, 2024; **Published:** November 27, 2024**Abstract**

Iodine deficiency disorders are a menace for the vulnerable Pakistani population for the past few decades. Universal Salt Iodization (USI) program was introduced to alleviate these issues. Effectiveness and sustainability of the program can be ensured through regular monitoring of salt iodization at various points of the supply chain. Traditional iodometric titration methods have problems related to accessibility, cost, and time. Colorimetric qualitative test kits are being used worldwide to estimate iodine content in salts during household surveys due to their expediency and affordability. Current study was planned by conceiving a similar idea of developing a spot test kit for qualitative analysis of salts in Pakistan. About 200 salt samples were collected and analyzed using kit and reference titration methods through single and multiple observers techniques. The trend of agreement between kit and titration values showed a sharp decline with an increase in the number of observers. Moreover, sensitivity of the kit was slightly higher for multiple observers (82.09% for single observer & 97.08% for multiple observers), but specificity declined sharply (69.7% for single observer & 40.4% for multiple observers). Low specificity and high false positives for multiple observers indicate that the kit might overestimate the availability of iodized salts, resulting in misleading information for program managers. Study findings highlight the role of iodine spot-testing using rapid kits and conclude that rapid kits can prove to be a valid and sustainable alternative option for quality assurance of food fortification standards in salts at all levels, from producer to consumer, to ensure effectiveness of the programme.

Keywords: Rapid Test Kit; Titration; Iodine Content; Validation; Colorimetry; Salt; Iodization**Introduction**

Pakistan is the 2nd world's largest and 1st Asian region salt producer while ranked 12th in salt exports. Rock, sea, table and lake salts produced in Pakistan are exported to different countries including USA, EU, China, Malaysia, Middle East, Brazil and Russian Federation (<https://agro.tdap.gov.pk/pakistan-salt>). Each type of salt possesses unique physico-chemical, nutritional and optical characteristics. Rock salt commonly labelled as pink Himalayan salt is the most natural form, retaining a variety of minerals and trace elements (potassium, calcium, magnesium, iron, and manganese), while table salt also known as common salt or kitchen salt is highly refined/processed granulated white salt and typically contains anti-caking agents to avoid clump formation and maintain brittleness. It is used during cooking or even directly added while eating a meal. Likewise, iodized salt is crystalline white (pale or pink) table salt with added iodine for dietary supplementation/fortification (www.egawellness.in/difference-between-regular-salt-and-himalayan-rock-salt; PPF, 2018). Iodized salt should be free from visible contamination with clay, grit and other extraneous impurities and contain potassium iodate (KIO₃) fortificant along with a stabilizer consisting of 0.1% sodium thiosulphate (Na₂S₂O₃) in order to prevent the loss of iodine. Iodine content in iodized salt should be between 30-50 parts per million (ppm) with the level of iodization fixed at a minimum of 15 ppm at the consumer level and 30 ppm at the production level (PPF, 2018).

Iodine is an essential micronutrient required by the body to produce thyroid hormones which control the body's metabolism and other vital functions. Its deficiency can cause a number of disorders often referred to as Iodine Deficiency Disorders (IDDs) including improper brain and bone development, intellectual capacity and growth of a person. Over the last few decades, universal salt iodization (USI) program has emerged as a major cost-effective strategic contributor to combat IDD [12] (The Government Gazette, 2011). Statistical analysis of national nutrition survey of Pakistan revealed that approximately 15.7% of children aged 6–12 years have low Urinary Iodine Excretion (UIE), with a slightly higher rate among girls (16.2%) than boys (15.2%). Similarly, about 17.5% of women of reproductive age (≈15 to 49 years) (WRA) have low UIE and among those, 12.9% showed moderate and 4.6% recorded severe deficiency (NNS, 2018-19).

Controlled mechanized monitoring and evaluation plan of national salt iodization program is necessary to harvest fruitful results and indicate the program's efficacy. Further, another important factor is rigorous assessment of iodine content in different salts to evaluate the adequacy of iodine concentration [2]. Traditional iodometric titration method has been used as a standard method for determination of iodine in salts. In spite of its accuracy, the method is pretty time-consuming and costly, requiring huge capital investment in terms of infrastructure and skilled manpower [11]. Contrarily, rapid/spot testing

kits & tools are simple, economical, easy-to-use and give instant, on-spot accurate qualitative and semi-quantitative results. Another eye-catching merit point of rapid kits is their portability which enables its usage in field inspection of fortifying agents [9]. Major objective of the current study was to develop & validate a rapid testing kit for qualitative and semi-quantitative assessment of iodine content (ppm) in different types of salts used in various geographical regions of Pakistan.

Materials and Methods

Procurement of Chemicals & Salt Samples

Analytical grade (AR) chemicals (H_2SO_4 , $Na_2S_2O_3$, Starch, KIO_3 , analytical grade NaCl & KI), prepared and packed by Dae-Jung Korea and imported by a local supplier Musa-Ji Adam & sons, Pakistan. Suppliers were bound to provide Certificate of Analysis (CoA) for each chemical and all the chemicals were pretested in laboratory for validation purposes prior to experimental use. Deionized water (diH_2O) was used in all experiments obtained from Thermo Scientific Deionizer (DI-425) installed in Food Nutrition Laboratory (FNL) (Room # 125), NIFA, Peshawar. Consumable stores like plastic bottles with locks, tips & caps, packing boxes, leaflets or instruction pages etc. were printed from local market of Peshawar.

Different salt samples (200 nos.) including table salt, rock salt was collected randomly from different locations of districts Peshawar & Nowshera, Khyber-Pakhtunkhwa (KP), Pakistan. Sample collection places were wholesale dealer shops/godowns, retailer karyana shops, local salt crushing plants & house hold consumers after mutual consent. Sea salt samples were collected by Nutrition International (NI), Pakistan from Karachi and its nearby places and sent to FNL for experimentation. Collected samples were kept in zip-lock clean poly-plastic bags inside light and moisture avoiding container/s at room temperature (20 ± 5) °C throughout the study. Samples were shifted to FNL, NIFA, [1] Peshawar for further analytical studies.

Preparation of Standard Iodized Salt Samples

Standard solutions of iodized salt samples were prepared by dissolving NaCl in D.I. water to obtain 20% solution. Suitable amounts of KIO_3 solution were added in NaCl solution to get final iodine concentrations of 0, 10, 20, 30, 40, 50 and 100 ppm respectively. Homogenous and fine salt crystals were prepared through lyophilization process. Quantitative analysis of iodized samples was carried out using standard iodometric titration method (20 samples of each concentration).

Iodometric Assay of Iodine Content in Iodized Salt Samples

Standard iodometric titration method was followed with slight modifications for quantitative assessment of standard iodized salt samples. Each sample was analyzed in triplicate to get accurate and precise results. Iodine concentration was converted into parts per million (ppm) by applying formula.

$$\text{Iodine Concentration (ppm)} = 10.58 * \text{Titer Reading}$$

This study set baseline for sample analysis of unknown concentrations and thus interpretation of the results to calculate exact quantity of iodine.

Optimization and Standardization of Kit Reagents

Developed rapid test kit was optimized for its reagent's concentration and chemical reaction parameters to enhance its applicability & reliability for all salt types with special focus on sea salt. As sea salt is more water saturated and stability of KIO_3 in this salt is very low due to the fact that KIO_3 tends to evaporate from sea salt immediately after its addition. Thus, the main point of concern was to optimize & standardize such concentration of kit reagents which will be equally effective for each type of salt sample irrespective of its composition or chemical & physical structure. A series of experiments was conducted on different salt types with varying (increasing/decreasing) concentrations of kit reagents until optimum results were achieved. If the tested sample showed "nil iodine (0 ppm)" on first attempt, the sample was acidified to neutralize the presence of alkali in the sample and retested. If the test still showed "no iodine", this was considered as the true test result. Based on the final results, it was concluded that the developed kit can also be used for semi-quantitative determination of iodine content in salt samples at various concentrations i.e. 0, 10, 20, and 30 ppm, depending upon the intensity of developed colour. The kits were stored under different storage conditions & used for at least twelve (12) months before marking the expiry date on packing box.

Determination of Iodine content in Salt Using Rapid Test Kit

Different number of salt samples were randomly selected from lot of collected samples for qualitative assessment of iodine content. Different data collection techniques were applied to gather data including single observer (well-trained/un-trained), multiple observer (well-trained/un-trained) and comparative analysis between the two identities [kit (well-trained/un-trained) and titration method (well-trained)]. Brief introduction and procedure to use the kit are outlined below.

Kit Apparatus

The field test apparatus consists of the following:

- Packing box containing labelled plastic bottles and instruction leaflet (01no.)
- One packing box contains (03 nos.) tightly capped milky white colored bottles of 10ml volume filled with reagent solution (Total Reagent Solution = $10*3 = 30ml$)
- Complete procedure of kit use is mentioned on instruction leaflet

Kit Procedure

- Taking 2-3 grams (approx.) powdered/grounded salt sample in a petri dish (if clumps/aggregates of salt sample are seen then ground it to fine powder)
- Making an even layer of sample by spreading it with the help of a wooden/SS spatula
- Stirring the reagent bottles gently before use
- Putting drop wise reagent solution on various spots of sample

- e) Waiting for a few seconds and letting the color develop
- f) Reading the result by comparing the developed color with color chart on packing box
- g) Estimating the iodine concentration (ppm) from color chart against the color intensity
- h) Recapping the bottles tightly & storing the kit properly to get maximum shelf life

Data Analysis

Obtained data were statistically analysed as described by Steel et al. 1997 using 2-way frequency table at 95% Confidence Interval (CI)/0.05 % level of significance (α). To validate the rapid test kit, different parameters like sensitivity, specificity, false positive & false negative rates were assessed. The main aim of monitoring USI was to estimate the availability of “adequately” iodized salt (≥ 15 ppm of iodine). Additionally, results data was also bifurcated into 02 categories & analysed according to the presence (>0 ppm) OR absence (0 ppm) of iodine in tested samples.

Results and Discussion

Need of Rapid Kit for Laboratory & field Testing

In most developing countries, salt is iodized with potassium iodate rather than iodide [1]. Keeping in view all the requirements and needs of lab & field testing, designing & development of rapid test kit was carried out. Kits were used inside and outside laboratory (field) by untrained personnel or personnel with limited training and generally lacking testing equipment & supplies. To universalize the kit effectiveness, all apparatus needed for testing must be self-contained inside the kit. In addition, kit should be easy-to-use, economical, portable, have long shelf life and most importantly produce instant on-spot results with acceptable accuracy & precision. Traditional laboratory analysis to check the kit efficacy must be followed up to ensure customer's reliance on the developed product.

Characteristics & Safety Measures of Developed Iodine RTK

To facilitate laboratory & field inspections, the said spot test kit was developed and validated against the standard iodometric method as well as for multiple observers. On an average, kit can analyze ≈ 150 samples and have a shelf life of one year from its date of manufacturing if stored properly under optimum conditions of temperature (20–25°C), R.H. (50–60%) and away from light & moisture. The kit can estimate the iodine content either qualitatively or semi quantitatively. By “Qualitative” we mean that the salt samples being tested can be classified as adequately iodized or un-iodized. Spot test does not give exact level of iodine in the analyzed salt sample. While some kits yield semi-quantitative results of iodine content in different salts. Due to their portability, kits can be easily transferred to far off places for field testing with little or no technical/scientific knowledge. Developed testing facility can assist relevant stakeholders like food regulatory bodies (food authorities in Pakistan), salt industry (salt crushing units/plants), international NGOs working on nutrition in Pakistan as well as the household consumers; in quality assurance & quality control through implementation & effective monitoring of food fortification standards in food supply chain. Besides monitoring,

rapid testing of iodine is one of the most powerful tools to advocate & educate the consumers about good health. Safety measures during kit use may include; using Personal Protective Equipment (PPEs) e.g. disposable gloves, safety goggles, face mask, hair & beard net etc. to avoid contamination, hazardous effects of kit and all potential risks which can compromise the quality of end results. Furthermore, in case of any non-conformities or non-compliances; proper mitigation strategies should be formulated and applied to minimize the residual risk associated with the potential risk factors. Finally, tested samples should be properly discarded and used glassware should be carefully washed & dried before next use.

Quality Control of Standard Iodized Salts

Standard iodized salts with various concentration ranging from 0 to 100 ppm were prepared by in-house method as detailed in materials and methods section. Spectrophotometric analysis was used to quantify iodine content in salt samples (20 nos.) from each concentration. Acceptable range was set at not more than $\pm 2SD$, at

Table 1: Quantitative assessment of iodine content in indigenously prepared standard iodized salts by iodometric titration analysis.

Theoretical iodine content (ppm)	Actual iodine content (ppm)	
	Mean \pm SD (n = 20)	CV (%)
0	0.03 \pm 0.00	0.00
10	10.05 \pm 0.14	1.39
20	19.62 \pm 0.35	1.78
30	28.98 \pm 1.02	2.24
40	41.02 \pm 1.05	2.56
50	50.96 \pm 1.45	2.84
100	98.23 \pm 1.85	1.88
Average Percent (%) Yield	97.66%	

Table 2: Comparison of kit testing against iodometric titration method for determination of iodine content in salt: Single-Observer Data.

Rapid Test Kit (ppm of iodine)	Iodine concentration by Iodometric Titration (ppm)			Total
	0	0.1–14.9	≥ 15	
0	1	17	5	23 (23.0) ^a
7	0	5	7	12 (12.0)
15 and 30	0	10	55	65 (65.0)
Total	1 (1.0) ^a	32 (32.0)	67 (67.0)	100

^aValues in parentheses are percentages

the precision level of $<5\%$ CV (Coefficient of Variation). Results of a single preparation lot are shown in (Table-1).

Single-Observer Data

A total of 100 (nos.) blind salt samples were analysed by a single observer and titration method. Both methods showed almost similar percentage of iodized salt samples with iodine content at ≥ 15 ppm (65.0% by kit and 67.0% by titration method) (Table 2). However, during qualitative assessment of iodine in salts, significant difference in false positive results was observed where kit was detecting 0 ppm iodine in salt samples but most of those samples did contain some level of iodine in them. It is evident from Table-2 data and such misleading information can result into complacency or false perception about iodization status thereby, raising serious questions on the effectiveness and efficiency of USI program. The sensitivity of the kit to detect adequate (≥ 15 ppm) and inadequate (<15 ppm) iodine content in salts was recorded at 82.09% (95% Confidence Interval (CI) = 79.3–85.4) and specificity was found to be 69.7% (95% Confidence Interval (CI) = 68.4–71.1). Sensitivity/Limit of Quantification (LOQ) is the minimum amount of the analyte (iodine in salt) that can be quantitatively determined with suitable precision and accuracy. While specificity/Limit of Detection (LOD) is the degree to which a method/testing tool can quantify/detect the specific analyte i.e.

Table 3: Comparison of kit testing against iodometric titration method for determination of iodine content in salt: Multiple-Observer Data.

Rapid Test Kit (ppm of iodine)	Iodine concentration by Iodometric Titration (ppm)			Total
	0	0.1–14.9	≥15	
0	1	8	1	10 (6.7) ^a
7	1	9	2	12 (8.0)
15 and 30	2	26	100	128 (85.3)
Total	4 (2.7) ^a	43 (28.7)	103 (68.7)	150

^aValues in parentheses are percentages

Table 4: Validation of iodine rapid test kit as qualitative testing tool/method.

Test Description	Validation Parameters (%)			
	Sensitivity	Specificity	PPV	NPV
Iodine present (> 0 ppm) vs. Iodine absent (0 ppm)	93.8 (91.4–95.2)	25.0 (0.2–30.8)	99.0 (98.2–99.8)	2.3 (0.2–9.5)
Multiple-observer data	77.8 (75.1–79.5)	100.0 (73.7–100.0)	100 (99.5–100.0)	1.8 (0.6–3.5)
Single-observer data				
Iodine adequate (≥ 15 ppm) vs. Iodine inadequate (< 15 ppm)	97.08 (95.4–98.6)	40.4 (37.2–42.9)	82.3 (77.8–85.7)	79.2 (65.3–85.3)
Multiple-observer data	82.09 (79.3–85.4)	69.7 (68.4–71.1)	89.5 (86.8–93.2)	93.7 (90.3–95.8)
Single-observer data				

Values in parentheses are at 95% confidence intervals

PPV = positive predictive value; NPV = negative predictive value

the micronutrient of interest (iodine) accurately in the presence of interferents. Correspondingly, the positive predictive value was 89.5% (95% CI = 86.8–93.2) while the negative predictive value was 93.7% (95% CI = 90.3–95.8) (Table 4).

Multiple-Observer Data

For multiple observer data, 150 (nos.) samples were analysed against kit and titration method and data was tabulated in Table-3. The ratio of samples having adequate iodine level (>15 ppm) was 85.3% using the kit against 68.7% by titration method. Data indicates that high number of false positives were shown by the kit due to overestimation of iodine content in analyzed samples. Spot-testing with multiple observers to check adequate and inadequate iodine contents displayed high sensitivity (97.08%) (95% CI = 95.4–98.6) but poor specificity (40.4%) (95% CI = 37.2–42.9). Positive & negative predictive values were 82.3% (95% CI = 77.8–85.7%) & 79.2% (95% CI = 65.3–85.3) (Table-4).

To strengthen the validation status of developed kit, salt samples were divided into two other groups as well: iodine present (>0 ppm) or iodine absent (0 ppm). Results obtained with these group divisions were quite similar to those already mentioned above with specificity and overall trend of agreement showed sharp decline for multiple observers in contrast to a single observer. However, number of uniodized salt samples were very few.

Current study revealed a sharp decline in agreement between the kit and the titration method for both types of assessments (qualitative and semi-quantitative) using multiple observer technique. Sensitivity of the kit in differentiating adequate (≥15 ppm) and inadequate (<15 ppm) iodine content of salt samples was alike for both single & multiple observers. However, rate of false positives was much higher for multiple observers than single observer which is due to the fact that the multiple observer work in realistic field conditions. Another bottleneck in using kits is that there is no clear demarcation line for

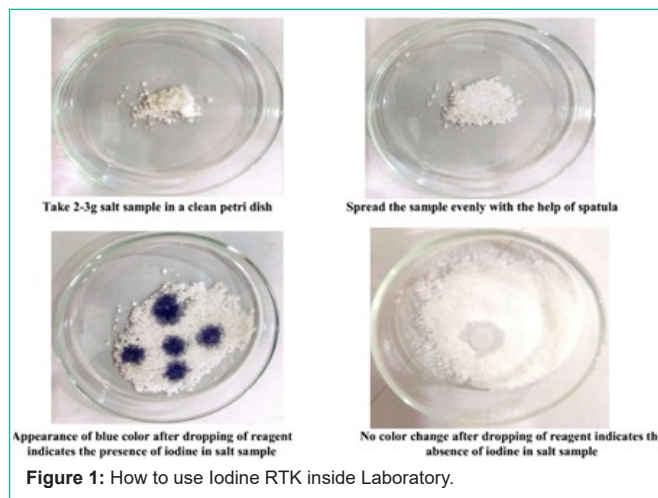


Figure 1: How to use Iodine RTK inside Laboratory.

declaration of results as it is qualitative in nature and the end results are always ambiguous which do not clearly describe the actual status of analyzed sample. For example, the color appearance on 10 ppm iodine content does not mean that it is exactly 10 ppm, it might be >10, ≈10 or falls within a specific range (8.2 – 14.5), all these situations successfully achieve the criterion for iodized salt sample/ positive result.

Therefore, while using the RTKs there is always a chance of mishandling the output information which needs to be cross examined by standard quantitative method. After validating the results quantitatively, we can measure the precision and accuracy of kit results. Findings of this study was found to be in comparison with those mentioned by Ounjajean et al. (2020) during development and validation of USI-Kit for evaluation of iodine content in iodized salt. Another study was conducted by Pandav et al. (2000) and achieved sensitivity (93.9%), specificity (40.4%), false positive (78.2%) & false negative (74.3%) results during validation studies of iodine spot test kit in two states of India using multiple observer technique.

Development of color Chart

During developmental studies on rapid test kit, one more feature was worked out that was the development of color chart which is based on the semi-quantitative reading of iodine content in a sample. Standard iodized samples of known concentrations i.e. 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 ppm along with a blank (0 ppm) were analysed with the kit to check the intensity of developed color and time taken to develop the color. After conducting several trials, a tentative color chart was developed under the supervision of trained experts to estimate the iodine content. As kit is qualitative in nature, so the developed color chart does not provide the exact level of iodization in the analysed sample rather it just gives a slight indication of level of iodine based upon the intensity of the color developed. Step-wise use of the kit inside laboratory (Figure-1), developed RTK (Figure-2) and color chart (Figure-3) are sketched at the end for ease of understanding for the readers.

Conclusive Remarks

These results show the performance of one specific kit that is used extensively in Pakistan. We supply these kits to salt industry, salt crushers, food regulatory authorities and INGOs working on



Figure 2: Iodine Rapid Test Kit.

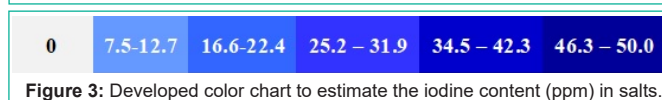


Figure 3: Developed color chart to estimate the iodine content (ppm) in salts.

nutrition in Pakistan. Although there are some other suppliers of such type of kit, but the results of those kits are not sustainable and precise. We have carried out a thorough R & D on the development of this kit and we still are doing our best to improve its accuracy and precision in terms of its sensitivity, specificity and color development index. But still, efforts should be made to improve the overall performance of all currently available kits. Our laboratory is also designated “Iodine Reference Lab” in Pakistan for determination of iodine contents in salts through standard titration method and we conduct an annual training session for this activity to train the health officials of Province Khyber-Pakhtunkhwa (KP), Pakistan. Conclusively, although Iodine RTKs for salt analysis are very useful components of public health programmes but until a valid alternative is available, the titration method should be used continuously for monitoring the iodine contents of salts during salt supply chain from its production sites through distribution points (retail shopkeepers) till household consumptions.

Author Statements

Ethics Approval and Consent to Participate

It is declared that the manuscript doesn't contain any unethical material and is allowed to participate.

Consent for Publication

It is declared that the manuscript is granted permission for publication in the journal.

Availability of Data and Materials

For any correspondence, data availability & materials requests, corresponding author may be addressed.

Competing Interests

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