Development and validation of a rapid test kit for iodine detection in salt

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Abstract

Iodine deficiency disorders are widespread among vulnerable populations in Pakistan. To combat this menace, the Universal Salt Iodization program needs regular monitoring throughout the salt supply chain. Traditional titration methods are limited by accessibility, cost, and time, making rapid test tools a more practical alternative for household surveys. In this context, the current study was designed to develop a rapid test kit for on-the-spot analysis of salt samples and validate it against standard reference methods by applying single- and multiple-observer study designs. Results indicated that the regression plot analysis of the developed kit produced an excellent average R^2 value of 0.9949. Percent yield/recovery of the titration method and the rapid test kit was achieved at 97.66% and 88.7-102.4%, along with a maximum value of coefficient of variance of 2.84 and 3.45, respectively. Moreover, the limit of detection/sensitivity was higher for multiple-observer designs (97.08%) as compared to the single-observer design (82.09%). The limit of quantification/specificity declined sharply for the multiple-observer design (40.4%) against the single-observer design (69.7%). This study revealed that despite yielding low specificity for multiple observers and occasional overestimations, the developed kit demonstrated strong potential as a sustainable and efficient alternative tool for iodine testing, thereby supporting and enhancing the overall impact of the salt iodization program in the country.

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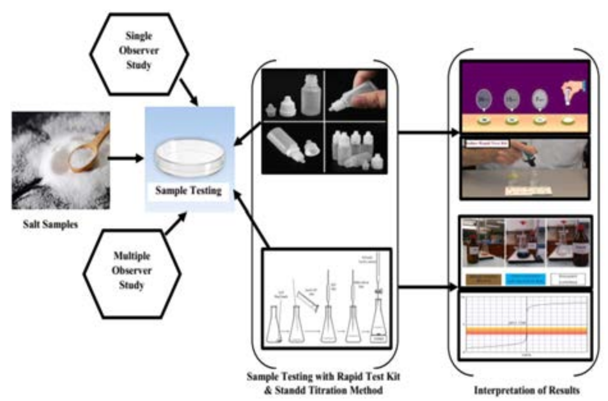

Graphical abstract.

Introduction

Pakistan is the second world's largest and the first Asian region salt producer, while it ranked 12th in salt exports. Rock, sea, table, and lake salts produced in Pakistan are exported to different countries, including the USA, the European Union, China, Malaysia, the Middle East, Brazil, and the Russian Federation (Trade Development Authority of Pakistan, 2024). Each type of salt possesses unique physico-chemical, nutritional, and optical characteristics. Rock salt, also referred to as pink Himalayan salt, is a pure natural salt, containing a variety of minerals and trace elements like potassium, calcium, magnesium, iron, and manganese. While table/common/kitchen salt is highly refined/processed granulated white salt and typically contains anti-caking agents to avoid clumping and maintain brittleness. It is used during cooking or sometimes directly added during eating meal. Similarly, iodized salt is a crystalline white (pale or pink) table salt with fortified iodine for dietary supplementation (EGA Wellness, 2024; Punjab Pure Food Regulations, 2018). Iodized salt should be free from visible contamination with clay, grit, and other extraneous impurities and may contain potassium iodate (KIO_3) as a fortificant along with 0.1% sodium thiosulfate ($Na_2S_2O_3$) stabilizer to prevent iodine loss. Further, iodized salt shall have 30-50 parts per million (ppm) iodine content, with a fixed iodization level at 15 ppm at the consumer level, while 30 ppm at the production site (Punjab Pure Food Regulations, 2018). Iodine is a vital micronutrient necessary to produce thyroid hormones and regulate body metabolism, along with other important bodily functions. Iodine deficiency can lead to multiple health problems referred to as iodine deficiency disorders (IDDs). These include impaired brain and bone development, reduced intellectual capacity, and stunted growth. Over the past few decades, the Universal Salt Iodization (USI) program has become a cost-effective and impactful strategy to combat IDDs (World Health Organization, 2007; Laksanawisith, 2011). Data from Pakistan's National Nutrition Survey (2018) highlights the prevalence of iodine deficiency among vulnerable population segments; ≈15.7% of children aged 6-12 years exhibit low urinary iodine excretion (UIE), with a slightly higher prevalence in girls (16.2%) compared to boys (15.2%). Among women of reproductive age (15-49 years), 17.5% have low UIE, with 12.9% showing moderate deficiency and 4.6% experiencing severe deficiency. A controlled, mechanized monitoring and evaluation plan is vital for the success and efficacy of the national salt iodization program. Accurate assessment of iodine levels in salt is equally important to ensure adequate concentrations (Rohner *et al*., 2015). While iodometric titration remains the standard quantitative tool for iodine determination, it is costly, time-intensive, and requires significant infrastructure & skilled personnel (Boonamsiri *et al*., 1976). In comparison, rapid spot-test kits are cost-effective, portable, and user-friendly, providing instant qualitative and semi-quantitative results, making them ideal for field inspections (Ounjaijean *et al*., 2020). In this context, the current study aimed to develop a rapid test kit (RTK) for on-spot, precise, and accurate analysis results with a main focus on cost-effectiveness, ease of use, acceptable limit of detection (LOD), and limit of quantification (LOQ) to comply with the government food fortification standards.

Materials and Methods

Procurement of chemicals and salt samples

Analytical grade chemicals (H_2SO_4, $Na_2S_2O_3$, Starch, KIO_3, analytical grade NaCl & KI), prepared and packed by Dae-Jung (Busan, South Korea), and imported by a local supplier Musa-Ji Adam & Sons (Peshawar, Pakistan). Suppliers were bound to provide a certificate of analysis for each chemical, and all the chemicals were pretested in the laboratory for validation purposes before being used for experimentation. Deionized water (diH_2O), obtained from Deionizer, model no. DI-425, made by Thermo Fisher Scientific at Stockland 3, D-56412 (Niederelbert, Germany), installed in the Food Nutrition Laboratory (FNL), Nuclear Institute for Food and Agriculture (NIFA), Peshawar, was used in all the experiments. Consumable stores like plastic bottles with locks, tips, and caps, packing boxes, and instruction leaflets were procured from the local market of Peshawar.

A total of 200 salt samples consisting of table and rock salt were randomly collected from Peshawar and Nowshera, KP, Pakistan, at wholesale shops, godowns, retail stores, salt crushing plants, and households. Sea salt samples, sourced by Nutrition International from Karachi and nearby areas, were sent to FNL for analysis. All samples were stored in zip-lock bags within light- and moisture-resistant containers at room temperature (20±5°C) and transported to FNL for further experimentation.

Preparation of standard iodized salt samples

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

Iodometric and spectrophotometric assay of iodine contents in iodized salt samples

Standard iodometric titration method and spectrophotometric method were followed with slight modifications for quantitative assessment and determination of the coefficient of correlation (R^2) of standard iodized (KIO_3) salt samples. Each sample analysis was replicated to get accurate & precise results. Iodine concentration was converted into parts per million (ppm) by applying Eq. 1 in the official methods of the Association of Official Analytical Chemists (AOAC, 1925):

$$\textbf{Iodine concentration (ppm)} = \frac{\text{Titration volume (ml)} * 21.15 * \text{Normality of sodium thiosulfate} * 1000}{\text{Salt sample weight (g)}}$$

[Eq. 1]

This study set a baseline for sample analysis of unknown concentrations and thus interpretation of the results to calculate the exact iodine quantity. Samples with absorbance values exceeding the value of 2.5 optical density threshold were diluted and subsequently re-analyzed. The respective dilution factors were then incorporated into regression plot (Figure 1).

Optimization and standardization of kit reagents

A RTK was optimized for its reagent concentration and chemical reaction parameters to enhance its applicability and reliability for all salt types, with special focus on sea salt. As sea salt is more water saturated and the 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 and standardize such a concentration of kit reagents, which would be equally effective for each type of salt sample, irrespective of its composition or chemical and 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 the first attempt, the sample was acidified to neutralize the presence of alkali in the sample and retested. If the test still showed "no iodine", it was considered 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 the developed color. The kits were stored under different storage conditions and used for 15 months before mentioning the expiry date on the packing box.

Determination of iodine content in salt using rapid test kit

Different numbers of salt samples were randomly selected from a 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)]. Selected observers produced a medical fitness certificate (color blindness) to differentiate color intensity among samples. A brief introduction and procedure for using the kit are stated below.

Kit apparatus

The field test apparatus consists of the following: i) packing box containing labeled plastic bottles and instruction leaflet (01 no.); ii) one packing box contains (03 nos.) tightly capped milky white colored bottles of 10 mL volume filled with reagent solution (total reagent solution volume = 10*3 = 30 mL); iii) complete procedure of kit use is mentioned on the instruction leaflet.

Step-by-step test kit application procedure

i) Take about 2-3 g of powdered/grounded salt sample in a petri dish (if clumps/aggregates of samples are present, then grind these into a smooth, even-sized fine powder particles).
ii) Make a smooth sample layer by spreading along the diagonal axis and across cross-sections with the help of a wooden/SS spatula for fine distribution of test sample particles.
iii) Stir the reagent solution bottles gently to ensure a homogeneous solution makeup.
iv) Put drop-wise reagent solution on different places/spots of the test sample.
v) Wait for a brief moment (approximately 2 to 3 seconds) to let the color develop properly.
vi) Read the result carefully by comparing the developed color with the color chart given on the lateral side of the packing box.
vii) Estimate the iodine concentration (ppm) by calculating it from the color chart slab *vs.* the color intensity.
viii) Handle the kit materials properly by tightly recapping the bottles and storing the kit in appropriate conditions (20±5°C temperature, 50-60% relative humidity (RH), and away from light, heat, moisture, microbes, dust, *etc*.) to achieve optimum shelf life.

Data analysis

The obtained data were statistically analyzed using a 2-way frequency table at a 95% confidence interval (CI)/0.05 % level of significance (α) (Steel and Torrie, 1960). To validate the RTK, different parameters like sensitivity, specificity, false positive & false negative rates were assessed. The basic purpose of monitoring USI was to estimate the availability of “adequately” iodized salt (≥15 ppm of iodine). Additionally, the results of the data were divided into 02 categories and analyzed according to the presence (>0 ppm) OR absence (0 ppm) of iodine in the tested samples.

Results and Discussion

Need for a rapid kit for laboratory and field testing

In most developing countries, salt is iodized with potassium iodate (KIO_3) rather than potassium iodide (Diosady *et al*., 1999). Keeping in view all the requirements and needs of lab and field testing, the design and development of an RTK were carried out. Kits were used inside and outside the laboratory (field) by untrained personnel or personnel with limited training and generally lacking testing equipment and supplies. To universalize the kit’s effectiveness, all apparatus needed for testing must be self-contained inside the kit. In addition, the kit should be easy-to-use, economical, and portable, possess a long shelf life, and most importantly, produce instant on-spot results with acceptable accuracy and precision. Traditional laboratory analysis to check the kit’s efficacy must be followed up to ensure the customer’s reliance on the developed product.

Characteristics of the developed kit

The RTK has been developed and validated against the standard iodometric method and can support both laboratory and field inspections. A single kit can analyze approximately 100 test samples and offers a 1-year/12-month bench shelf life if stored properly as recommended in the instruction leaflet enclosed within the kit box. Kit must be kept at 20-25°C temperature with 50-60% RH

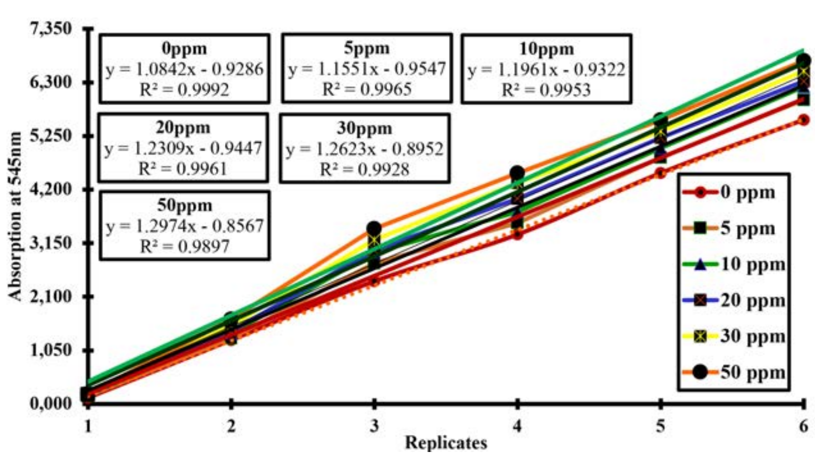

Figure 1. Regression plot of comparison between iodine concentrations and spectrophotometric absorptions.

and away from light, heat, and moisture to get maximum shelf life and reliable test results. The mentioned storage conditions are easy to meet inside the laboratory, while during field inspections, operators must ensure to maintain these parameters using portable hot/cool technologies to achieve optimum shelf life and results. The kit offers qualitative or semi-quantitative iodine estimation in salt. The qualitative test classifies samples as adequately iodized or uniodized, while some kits provide semi-quantitative iodine content. Kits are suitable for remote field testing due to their portable nature and easy-to-use structure with minimum technical knowledge. Such a rapid testing facility can facilitate the relevant stakeholders, including food regulatory bodies, the salt industry, international non-government organizations (INGOs) working on nutrition, and consumers, in ensuring quality control and monitoring food fortification standards. It can also serve as an effective tool for advocacy and health education. Safety precautions before, during, and after kit usage must be observed and implemented strictly to avoid any chances of contamination or non-compliance. Preventive measures may include the use of personal protective equipment, *i.e.*, hand gloves, safety goggles, face mask, beard mask, jewelry removal, and hair net. Besides, components of the kit may be handled and discarded by specialists with utmost care post-analysis. In case of any non-compliance, mitigation measures should be applied at once to reduce the hazardous risks associated with these incidents. Used samples should be properly discarded, and glassware cleaned before reuse.

Quality control of standard iodized salts

Standard iodized salts with various concentrations ranging from 0 to 100 ppm were prepared by an in-house method as detailed in the Materials and Methods section. The iodometric titration method was used to determine the iodine content in salt samples (20 nos.) from each concentration. Acceptable range was set at not more than ±2 Standard Deviation (SD), at the precision level of <5% coefficient of variation (CV). Results of a single batch are shown in Table 1.

Spectrophotometric analysis of iodized and non-iodized salt samples was conducted at iodine concentrations of 0, 10, 20, 30, and 50 ppm. The resulting calibration curves, shown in Figure 1, demonstrate the correlation between iodine concentration and absorption at 545 nm, as outlined by Mary *et al*. (2008) and Mix (1944). These curves demonstrate that with the increase in iodine concentration, absorption also increased in direct proportion, producing a strong CV (R^2=0.9949). R^2 value shows perfect correlation b/w iodine concentration and corresponding absorption, thereby supporting the development of a tentative color chart.

Validation by single-observer data

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

Table 1. Quantitative assessment of iodine in standard iodized salt samples by the reference titration method.

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

SD, standard deviation; CV, coefficient of variation

Table 2. Comparison of test kit results against titration method: single-observer data.

Rapid test kit (ppm of iodine)	Iodine concentration by iodometric titration (ppm)			Total
	0	0.1-19.9	≥20	
0	1	17	5	23 (23.0[a])
10	0	5	7	12 (12.0[a])
20 to 30	0	10	55	65 (65.0[a])
Total	1 (1.0[a])	32 (32.0[a])	67 (67.0[a])	100

[a]Values in parentheses are percentages (%)

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).

Validation by multiple-observer data

For multiple observer data, 150 samples were analyzed against the kit and titration method, and the data were tabulated in Table 3. The ratio of samples having adequate iodine level (>15 ppm) was 85.3% using the kit against 68.7% by the titration method. Data indicate that a high number of false positives were shown by the kit due to overestimation of iodine content in the 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 and negative predictive values were 82.3% (95% CI=77.8-85.7%) and 79.2% (95% CI=65.3-85.3) (Table 4).

To strengthen the validation status of the 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 groups were almost similar to the previously mentioned findings, where specificity and overall trend of agreement showed a sharp decline for multiple observers, in contrast to a single observer. However, a very small quantity of uniodized salt samples was observed during analysis using the kit. Application of the test kit in the field settings was much more challenging due to multiple barriers like user perception, acceptance and feedback, awareness, environmental conditions, and the type of samples, *etc*. All these parameters were preempted and well negotiated during the field use of the kit. Users were preaddressed about the advantages of the kit and its easy-to-use approach. They were informed that the kit can assist in improving the end product quality and aid in the accomplishment of regulatory and statutory requirements for food fortification. Potential end users of the kit were equipped with hands-on training and technical knowledge of the kit and its storage under different environmental conditions, *i.e.*, temperature, RH, heat, light, moisture, *etc*. For example, if a sample of rock salt shows a moisture content of more than 8% then it must be dried before the kit analysis for optimum results. Similarly, if the percent moisture of the sea salt sample is greater than 20%, it should be dried too before applying the kit to get accurate results. Collectively, all possible problematic factors that may affect the kit's performance were discussed in detail with positive feedback from kit users.

The 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 a multiple observer technique. Sensitivity of the kit in differentiating adequate (≥15 ppm) and inadequate (<15 ppm) iodine content of salt samples was similar for both single and multiple observer data. However, the number of false positives was much higher for multiple observer data than for single observer data because multiple observer data was collected against actual field conditions. Data collection in the field is very difficult to contain and manage, and is compiled after testing on commercial salt samples. Test results are likely to be hindered by some foreign substances or contaminants, including moisture, heat, cold, light, air, dust, and other intrinsic impurities in test samples, *etc*. Strict precautionary measures during field testing using the kit can reduce the chances of false negative results, mainly due to potential interfering agents. Further bottleneck in using kits is that there is no clear demarcation line for 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 the 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 fall within a specific range (8.2-14.5), all these situations successfully achieve the criterion for iodized salt sample/ positive result.

Supplementary Figure 1 presents single observer data compar-

Table 3. Comparison of test kit results against titration method: multiple-observer data.

Rapid test kit (ppm of iodine)	Iodine concentration by iodometric titration (ppm)			Total
	0	0.1-19.9	≥20	
0	1	8	1	10 (6.7a)
10	1	9	2	12 (8.0a)
20 to 30	2	26	100	128 (85.3[a])
Total	4 (2.7[a])	43 (28.7[a])	103 (68.7[a])	150

[a]Values in parentheses are percentages (%)

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

Test description	Validation parameters (%)			
	Sensitivity	Specificity	PPV	NPV
Iodine present (>0 ppm) *vs.* iodine absent (0 ppm)				
Multiple-observer data	93.8 (91.4-95.2)	25.0 (0.2-30.8)	99.0 (98.2-99.8)	2.3 (0.2-9.5)
Single-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)
Iodine adequate (≥15 ppm) *vs.* iodine inadequate (<15 ppm)				
Multiple-observer data	97.08 (95.4-98.6)	40.4 (37.2-42.9)	82.3 (77.8-85.7)	79.2 (65.3-85.3)
Single-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)

Values in parentheses are at 95% confidence intervals; PPV, positive predictive value; NPV, negative predictive value.

ing iodine analysis results from the kit and the titration method. The data highlights the kit's true positive results against the standard quantification method. Overall, the kit performed satisfactorily in detecting iodine content across different concentrations, as shown in the graphical lines. A few exceptions may be due to improper kit use or observer error. In conclusion, the kit accurately detects iodine levels in most samples with good repeatability and reproducibility. As the kit is qualitative in nature, it helps in the binary test results of either the presence or the absence of iodine in the tested samples. However, validation parameters showed that the kit had acceptable values for LOD (≥3.8ppm) and LOQ (≥7ppm), respectively.

When using RTKs, there is always a risk of mishandling the output, which should be cross-checked with standard quantitative methods. Once the results are validated quantitatively, the precision and accuracy of the kit can be assessed. The findings of this study align with those of Ounjaijean *et al*. (2020), who developed and validated the USI-kit for evaluating iodine content in iodized salts. Similarly, Pandav *et al*. (2000) reported sensitivity (93.9%), specificity (40.4%), false positives (78.2%), and false negatives (74.3%) during the validation of an iodine spot test kit in two Indian states using a multiple observer technique. Similar validation results were reported by Jamilan, Norizan, *et al.* (2025) during the development and validation of an on-site salt iodine detector kit as an alternative tool for salt iodization monitoring in Malaysia. The kit showed linearity (10.0-50.0 ppm, R^2=0.9956) with a LOD of 3.6 mg/Kg, and method recovery of 89.5-99.4%. Another study was conducted by Jamilan, Hussain *et al*. (2025) to check the feasibility of the Salt Iodate Micro-Method Reagent detection kit for salt iodate analysis in Malaysia. Validation results of the kit yielded linearity of 5.0-60.0 ppm, LOD at 6.8ppm, and percentage recovery from 93.0 to 108.3%. Whereas repeatability, intermediate precision, and reproducibility achieved a mean CV of 5.3%, 6.8%, and 5.9% respectively.

A similar study design was proposed by Rao and Ranganathan back in 1985 to develop a simple field kit for testing iodine in salt samples across India to strengthen the national salt iodization programs. The kit was supposed to be effective for both iodide and iodate-fortified salts. Moreover, kit ingredients, chemistry, mode of action, and development of the color chart were perceived in the same pattern as has been done in the current study. Another kit-oriented experiment was planned by Nepal *et al*. in 2013 to estimate household salt iodine content using RTKs and iodometric titration methods. This cross-sectional study was carried out on school children of seven randomly chosen schools from four districts of eastern Nepal. The RTK method showed sensitivity of 84.8% (82.0-88.0), specificity of 68.3% (59.0-77.0), a positive predictive value of 92.7% (92.0-94.0), and a negative predictive value of 48.6% (40.0-57.0) as compared to the values of the iodometric titration, respectively. Yadav *et al*. (2015) assessed the validity of a new portable device, iCheck Iodine, developed by BioAnalyt GmbH (Teltow, Germany) to estimate the iodine content in salts. The correlation coefficient between measurements by the two methods was obtained at 0.934, and the correlation coefficient between measurements using 1 g of iodized salt and 10 g of iodized salt by the iCheck Iodine device was 0.983.

Color chart to estimate iodine concentrations

A descriptive and self-explanatory color chart was established for semi-quantitative estimation of iodine content during the development and validation of RTK. For this purpose, a series of standard iodized samples with known concentrations (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 ppm) along with blank (0 ppm) samples were repeatedly tested to judge the color intensity and development time. Intensity of the color was observed and adjudged by an expert panel of trained scientists and laboratory staff. After conducting several trials, a duly approved and proofread color chart was chalked out by a trained scientific panel. Since the kit is qualitative in nature, the provision of a color chart does not guarantee the exact iodine amounts in the tested sample, but it aids in identifying the estimated iodine concentration in a sample based on its color intensity. Figure 2 and *Supplementary Figures 2-4* display different phases of the research study, including kit development, validation, laboratory use of the kit, and color chart, respectively.

These results signify the performance of a widely used kit in Pakistan, which FNL is supplying to multiple stakeholders like the salt-producing industry, salt crushers, food regulatory authorities, and INGOs working on malnutrition in Pakistan. Although there are some other suppliers who offer similar kits but the results lack accuracy, repeatability, and reproducibility. In comparison, the kit developed by NIFA, Peshawar, provides accurate, precise, on-spot, and valid test results due to comprehensive Research & Development efforts that have been undertaken during its development and validation. However, continual improvement is still underway to improve kit accuracy, sensitivity, specificity, and the color development index chart. Commercial production of the kit, especially in areas with low resources, may be difficult to manage and sustain because initially, it requires infrastructure, manpower, equipment, and other miscellaneous utilities. But once the facility is established, even on a small scale, its production can be upgraded easily. However, there are certain key challenges associated with the use of the subject kit, especially during field deployment, which can halt or alter the end result. These may include the type of sample, chemistry of the sample, fitness of the eye-sightedness of the observer, and topography and environmental factors of the area where the kit is being employed. So, these limitations must be kept in mind while using the kit in field settings, and necessary precautions may be taken to avoid any negligence or non-compliance in test results. Therefore, extra efforts are required to improve the overall performance & productivity of available kits for iodine testing in salt samples.

FNL of NIFA Peshawar has been designated as the Regional Iodine Reference Lab in Pakistan for the determination of iodine content in salt. The laboratory is also providing human resource development services to various departments of the government and private sectors by conducting annual refresher training courses. Additionally, the laboratory is also acting as a focal center for the supply of iodine RTKs throughout the country through INGOs working on the theme to mitigate malnutrition in Pakistan. Conclusively, iodine RTKs can be used as valuable analytical tools in public health programs and serve as a sustainable and viable alternative to old conventional methods. However, kit results should be cross-examined regularly against standard reference methods throughout the salt supply chain, from production to

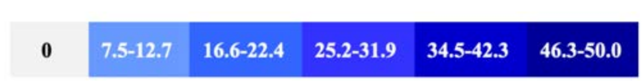

Figure 2. Color chart to estimate iodine (ppm).

household consumption, to ensure validity and precision. Furthermore, to evaluate the real-time shelf life of the developed test kit under various topographical conditions, kits should be stored at different geographical regions under varying storage parameters particularly in the regions with high humidity like along the coastal lines of Sindh province or in the areas with extreme/harsh weather conditions, *i.e.*, high temperature, dry air, low humidity *etc*. as in Southern Punjab, Interior Sindh or Baluchistan.

Conclusions

The current study highlights the effectiveness of an RTK as a reliable testing tool for the qualitative assessment of iodine in salts. Strong R^2 between iodine concentrations and colorimetric responses confirms its scientific validity. High sensitivity, ease of use, and cost-effectiveness reinforce the practical value of the kit for routine monitoring, particularly in field conditions where titration methods are not feasible. Despite some limitations in specificity, especially under multi-observer data compilation, regular validation against standard iodometric methods can improve the accuracy of end results. Conclusively, application of such on-spot test kits can support the regulatory compliance with iodization standards, contribute to early detection of inadequately iodized salts in the supply chain, and play a vital role in the prevention and control of IDDs, thereby revitalizing national public health interventions.

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Online supplementary material
Supplementary Figure 1. Rapid testing using kit against titration analysis for iodine determination in salts.
Supplementary Figure 2. Validation of the rapid test kit.
Supplementary Figure 3. Use of the developed kit inside the laboratory.
Supplementary Figure 4. Iodine rapid test kit.