

Evaluating the Effectiveness of the Rapid Test Kit Used in the Field Monitoring Of Iodine Content of Salt in the Philippine Market

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Abstract— With its ease of use and the minimal training required, the Rapid Test Kit (RTK) has been found to be suitable for field monitoring of compliance to government programs promoting salt iodization in the Philippines and in other countries. The test consists of adding two drops of the test solution on the surface of the salt and comparing the resulting color with the color chart included in the kit.

It has been shown that the colors in the accompanying chart simply do not match with those observed when test solutions are applied on the samples. The tests resulted to colors representing different shades of purple while the existing color chart in RTK presents shades of gray. A scoring system based on different shades of purple was used to quantify the results and allow the conduct of statistical analysis.

To further evaluate the effectiveness of the RTK to distinguish the different levels of iodization in the salt i.e. non-iodized (5ppm and below), inadequately iodized (10, 15, 20 and 25 ppm) and adequately iodized (30, 35 and 44ppm), experimental design and measurement system capability (Attribute Gage Repeatability and Reproducibility or Gage R&R) analyses were undertaken.

Results from the experimental design show that the type of salt used -- whether Local Solar, Imported Solar, Pure Dried Vacuum (PDV) or Cooked Salt – do not affect the resulting color (score) when the test solution is applied. There is no 'type of salt effect' and only the amount of iodine in the salt contributes to the variation in the shades of purple observed.

Further, the tests showed that inspectors had difficulty assessing samples within the inadequately iodized range. Iodine concentration levels at the lower rung of this range tend to be misclassified as 'poorly iodized' while those at the upper end of the range tend to be judged as 'adequately iodized'.

From the results of the experiments, three Reference Charts (5 ppm, 30 ppm and chart containing both colors for 5 ppm and 30 ppm) were proposed to replace the existing ineffective chart. These charts were evaluated in their ability to discriminate between the different levels of salt iodization.

Of the 3 proposed Reference Charts tested, the one involving one color only for 5 ppm is the most effective i.e. high percentage of agreement between inspectors on their judgements and that their judgements match the actual iodine concentration levels.

If the government will continue to use the RTK measurement system, the reference chart for one color for 5 ppm is the preferred replacement for the existing chart. However, its limitation should be recognized in that it can only confidently state that a salt sample is either 'poorly iodized' or *at the very least*, inadequately iodized.

Keywords— *Rapid Test Kit, Measurement System Capability, Salt Iodization*

I. INTRODUCTION

Republic Act No. 8172, otherwise known as “An Act Promoting Salt Iodization Nationwide and For Related Purposes”, mandates the Department of Health (DOH) and other government agencies to implement the Salt Iodization Program as a means to eradicate Iodine Deficiency Disorders (IDD) [1].

The objective of the program is to ensure that 90% of all households use iodized salt, a salt that has been added with iodine in the form of potassium iodate. One of the program strategies employed is salt testing to ensure that only iodized salt is sold in the market and is used for household consumption.

The initial objective of the program was to increase the availability of iodized salt, that is, salt added with any amount of iodine. In the most recent years, the program had emphasized that added iodine should be in conformity with the government standard of 30-70 ppm from production to retail levels, to ensure that salt reaches the household at 15 ppm, taking into consideration possible losses along the distribution channel. Monitoring at the market, household and other critical areas are important to ensure that only iodized salt is used for human consumption [2].

A Rapid Test Kit (RTK), a qualitative tool, is used to determine the presence or absence of iodine in salt. A single kit includes two 10-ml bottles of the test solution, one 10 ml bottle of recheck solution, 1 small white cup and a color chart.

The test consists of adding two drops of the test solution on the surface of the salt and comparing the resulting color with the color chart included in the kit. The matching with the color chart only provides an approximation of the amount of iodine present in the salt. Nonetheless, its ease of use and the minimal training required makes the RTK suitable for field monitoring of compliance to government programs promoting salt iodization, not only in the Philippines but also in other countries.

As such, evaluating its effectiveness and coming up with recommendations to improve its capability in detecting and approximating the iodine content of salt in the market and at the household levels will be beneficial in the monitoring and promotion activities of concerned government agencies and other stakeholders.

II. OBJECTIVES AND SCOPE OF THE STUDY

The main objective of the study is to evaluate the capability of the RTK to distinguish the different levels or extent of iodization in the salt: non-iodized (5ppm and below), inadequately iodized (10, 15, 20 and 25ppm) and adequately iodized (30, 35, 44 ppm).

The study also looked into whether the different types of salt have an effect on the resulting color after the application of the test solution. The types of salt used in the study are as follows: Local Solar, Imported Solar, Cooked Salt and Pure Dried Vacuum (PDV).

Different color charts were also proposed to determine which of them results to higher level of agreement in judgement among inspectors (i.e. inspector's own judgement and his judgement compared with others) and that of the reference value (in ppm).

III. METHODOLOGY OF THE STUDY

To achieve the objectives of the study, two (2) sets of experiments were performed: a Design of Experiment (DOE) study and a Measurement Capability Study (Attributes Gage Repeatability and Reproducibility Study or Gage R&R).

The DOE study utilized the Randomized Complete Block Design (RCBD) model to determine 'type of salt' effect. While the primary interest is on determining the ability of the RTK to distinguish among the iodine levels, RCBD can also determine if the effect of a block factor (salt type) on the resulting color is also significant. The concentration of iodine in the salt served as the Single Factor of interest, with 8 levels (in ppm) specifically: 5, 10, 15, 20, 25, 30, 35 and 44 ppm. Meanwhile, the different types of salt used (Local Solar, Imported Solar, Pure Dried Vacuum or PDV and Cooked Salt) served as Blocking Factor [3].

The RCBD model entails that one (1) observation per combination of the iodine concentration level (8 levels) and type of salt (4 types) be undertaken, for a total of 32 observations. Specifically, the following questions/concerns were addressed by the output of the model:

- a) Using RTK, is there a significant difference in the test results produced for the given iodine levels belonging to Non-iodized, Inadequately Iodized and Adequately Iodized salt classification? Which particular levels are the same and which ones, if any, are significantly different?*

From the results, the ability of the RTK measurement system to discriminate between the various levels of iodine concentration was determined.

- b) Do the test results differ as a result of differences in the type of salt used?*

The results show whether the outcome of the RTK test was affected by the different types of salt used.

- c) Average color per level of iodine concentration*

From the results, corresponding color measures pertaining to levels of iodine concentration were determined and a comparison with existing color chart was made.

Using the existing color chart (with three reference colors) and the proposed color charts generated from the DOE study, a gauge repeatability and reproducibility study (gauge R&R) involving samples from selected levels of iodine concentration was conducted. The four (4) selected iodine levels were as follows: 1 Level for Non-iodized (5 ppm), 2 different Levels from Inadequately Iodized (10 ppm and 20 ppm) and 1 Level from Adequately Iodized (30 ppm). Specifically, the following questions/concerns were addressed by the Gage R&R study:

- a) Using the RTK, will the same inspector, measuring the same salt sample get the same reading or judgement every time i.e. repeatability?*

- b) Using the RTK, will different inspectors, measuring the same salt sample get the same reading or judgement every time i.e. reproducibility?
- c) Using the RTK, will the inspectors' judgement match the standard or the reference value (i.e. the visual judgement reflect the true iodine content level in ppm) every time?

This attribute gauge R&R study is an experiment where each of the inspector will assess every sample three times, for a total of 60 observations for both operators. With 4 levels of iodine concentration and 3 Reference Color charts, 12 of these tables were generated [4,6].

Originally, 4 Reference Color charts were to be used i.e. the 3 charts generated from experimental design and the existing color chart. The existing color chart was not utilized due to mismatch in the observed color in the tested salt against colors reflected in the accompanying RTK chart.

For the experimental units used in the study, four (4) sets of non-iodized salt namely, (1) Local Solar (2) Imported Solar (3) Cooked and (4) Pure Dried Vacuum (PDV), were fortified with iodine using potassium iodate. Imported solar salt and PDV were sourced from an importer while the local solar and the cooked salt came from a local market and salt refinery in northern Philippines, respectively.

Target concentrations for each type of salt were 5, 10, 15, 20, 25, 30, 35 and 40 ppm. A 100 mL of 1000 ppm iodine was prepared by dissolving 0.1691 g of KIO₃ with distilled water. Different amounts of this solution were pipetted into the salts contained in respective plastic bowls/containers to attain corresponding target levels of iodine. All in all, 32 sets of fortified salts were prepared. After dispensing the required volume of iodate solution into the salt, each container was covered, and swirled and shaken several times to homogenize the sample.



Figure 1. Some of the Plastic Containers With the Prepared Samples

These fortified samples were used to evaluate the capability of the RTK. Prior to the RTK test, the actual concentration of iodine added to the salt was verified using the WYD Iodine checker. Fortification was carried out using the table below and undertaken on a per ppm level:

Table 1. Volume of KIO₃ Added to Achieve Target Fortification

Target ppm level	Volume of 1000 ppm KIO₃ added	Estimated ppm prepared
5	0.25	5
10	0.5	10
15	0.7	14
20	1.0	20
25	1.2	24
30	1.5	30
35	1.7	34
44*	2.2	44

*initial target was 40 ppm

Initial attempts to introduce the fortificant as a powder to a 50-g salt proved to be difficult. KIO₃ powder was heavy (density: 2.93 g/mL); the highest ppm level, 44 ppm, requires only specks of dust of KIO₃. Thus, the move to introduce KIO₃ as a solution.

The 85% to 115% acceptable recovery of fortificant added later helped to establish the range of levels of iodine which will be used to evaluate the capability of the RTK to effectively distinguish different levels as expressed by different color intensity and/or hue.

Table 2. PPM vs Acceptable Recovery

ppm	Acceptable Recovery, in ppm
5	4.2 - 5.8
10	8.5 - 11.5
15	12.8 - 17.2
20	17 - 23
25	21.2 - 28.8
30	25.5 - 34.5
35	29.8 - 40.2
44	37.4 - 50.6

IV. DISCUSSION OF THE RESULTS OF THE EXPERIMENTS

Initial applications of the test solution to salt samples showed that the resulting colors refer to different shades of purple – far different from the shades (grayish) presented in the accompanying color chart of the Rapid Test Kit.

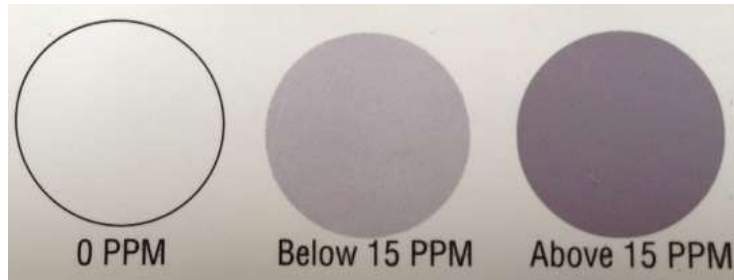


Figure 2. Existing RTK Color Chart

Translating the observed purple shades to numerical equivalents to allow statistical analysis initially presented a challenge in this study. Quantifying the different shades was made possible by using the different shades of purple below [5]. As the shades are presented from lightest to darkest (left to right), an arbitrary scoring system starting from 5 and increasing by five, with the highest at 70, was used. This chart and the generated scoring system were then used in the experimental design study.

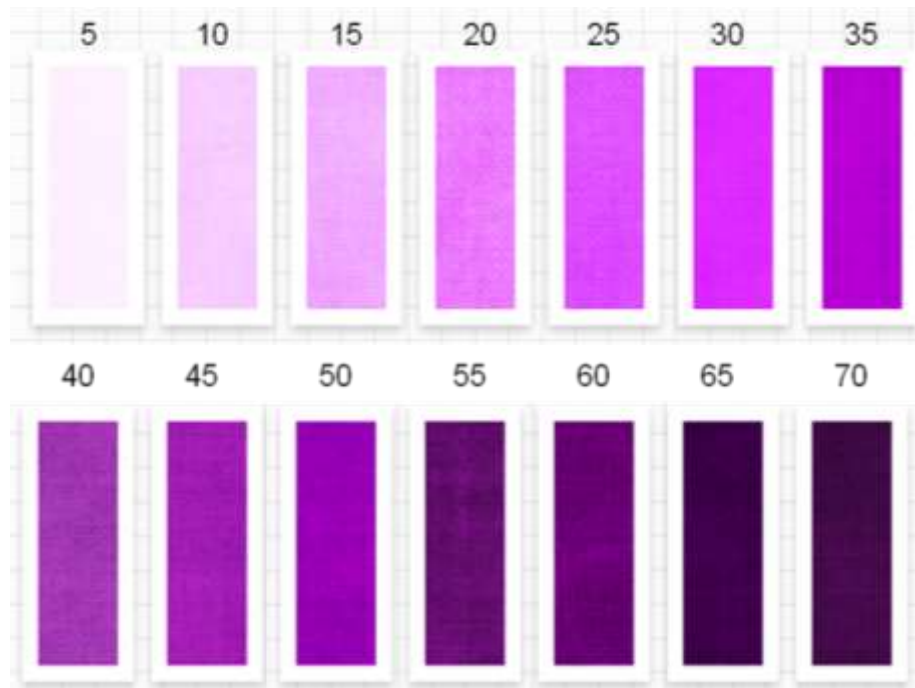


Figure 3. Appropriate Color Chart to Match Observed Colors on Tested Salt Samples

The randomized complete block design (RCBD) experiment was run with the following results with Iodine Content as the factor of interest and with 8 treatment levels (from 5 ppm to 44 ppm) and the four (4) types of salt (local solar, imported solar, PVD and cooked salt) serving as elements of the block.

Table 3. Data Table for the RCBD Experiment

Iodine Content (in PPM)	Score Based on the Color Chart			
	Local Solar	Imported Solar	Pure Dried Vacuum (PVD)	Cooked Salt
5	5	15	15	15
10	15	5	55	20
15	40	10	60	30
20	25	55	50	25
25	30	65	65	40
30	55	40	35	40
35	50	60	20	45
44	40	45	60	45

Figure 4 shows the samples prepared (left) for the experiment and the results upon application of the test solution (right)

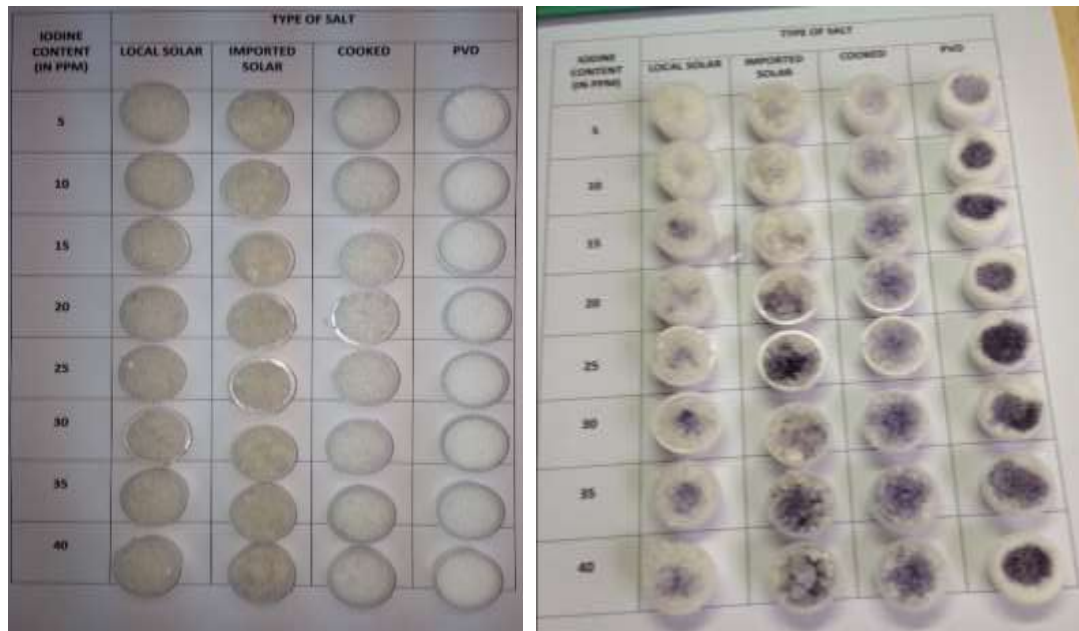


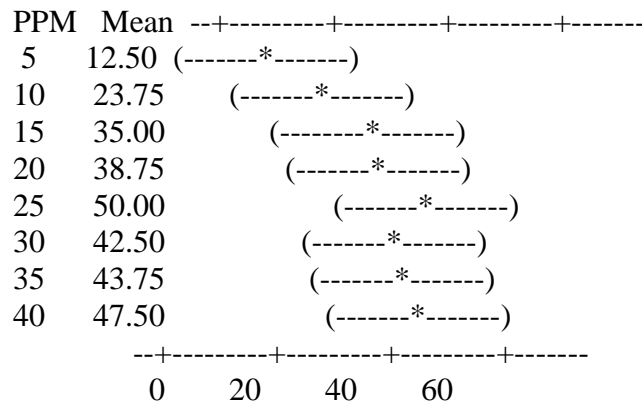
Figure 4. Prepared samples (left); Same Samples After Application of Test Solution

Below is the analysis of variance (ANOVA) output of the experiment using Minitab statistical software (using $\alpha=0.05$):

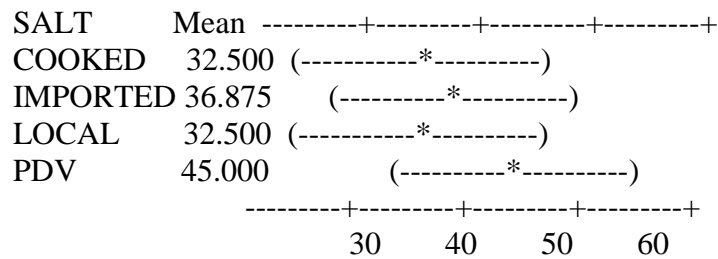
Two-way ANOVA: COLOR versus PPM, SALT

Source	DF	SS	MS	F	P
PPM	7	4549.2	649.888	2.73	0.035
SALT	3	833.6	277.865	1.17	0.346
Error	21	4997.7	237.984		
Total	31	10380.5			

Individual 95% CIs For Mean Based on Pooled StDev



Individual 95% CIs For Mean Based on Pooled StDev



Grouping Information Using Fisher Method

PPM	N	Mean	Grouping
25	4	50.00	A
40	4	47.50	A
35	4	43.75	A B
30	4	42.50	A B
20	4	38.75	A B
15	4	35.00	A B C
10	4	23.75	B C
5	4	12.50	C

Means that do not share a letter are significantly different.

Fisher 95% Individual Confidence Intervals

All Pairwise Comparisons among Levels of PPM

Figure 5. Analysis of Variance (ANOVA) Results using Minitab

- a) at $p=0.035$, we **Reject** the Null Hypothesis that there is no significant differences among the different iodine concentration levels, based on the resulting color change when test solution was applied. Simply stated, the different levels of iodine concentration in the salt samples result to different shades of purple, upon application of the test solution. This only means that resulting color change (score) in salt samples containing 5ppm and 35ppm of iodine, for example, are significantly different. This can also be confirmed from the plot of the Individual 95% CIs For Mean Based on Pooled StDev for the different ppm's where some confidence intervals do not overlap with the others (noticeable between results for poorly iodized and adequately iodized samples)
- b) On the contrary, at $p=0.346$, we **Do Not Reject** the Null Hypothesis that there is no significant differences among the different types of salt as far as the effect of the different iodine concentration levels is concerned. This means that a tester's assessment of the resulting color (score) when you apply the test solution to samples of different salt types containing only 5ppm of iodine, for example, do not vary – statistically speaking. This finding is likewise confirmed from the Individual 95% CIs For Mean Based on Pooled StDev for the Salt Types where the confidence intervals largely overlap with each other.

While ANOVA points to significant differences in the results for various iodine ppm levels upon application of the test solution, it should also be noted that based on the Grouping Information Using Fisher Method, certain treatments (ppm levels) do not differ significantly. As an example, results for treatment levels 5,10 and 15 are statistically the same (the C's). The results for levels 15-40 ppm also do not vary from each other (the A's). These observations point to difficulty in distinguishing resulting color changes for levels representing inadequate to adequate iodization.

The experiment fairly established the clear distinction between results for poorly iodized salts and adequately iodized salts. However, results for inadequately iodized salts (especially at lower levels of 10 and 15) overlap with that of poorly iodized and adequately iodized salts. In practical terms, this

observation suggests that it is not easy for inspector to score appropriately and correctly range of levels for inadequately iodized salts.

From the ANOVA, the mean scores for ≤ 5 ppm and 30ppm are 12.50 and 42.50 respectively.

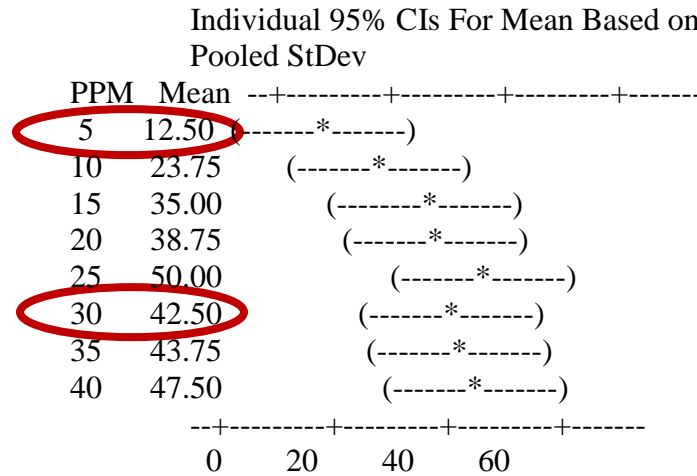


Figure 6. Mean Scores for ‘Poorly Iodized’ and ‘Adequately Iodized’ Salt

With reference to the color chart (featuring various shades of purple) used in the experimental design, the mean scores of 12.50 (for 5 ppm) and 42.50 (for 30 ppm) conservatively pertain to the shades with scores of 10 and 40 respectively.

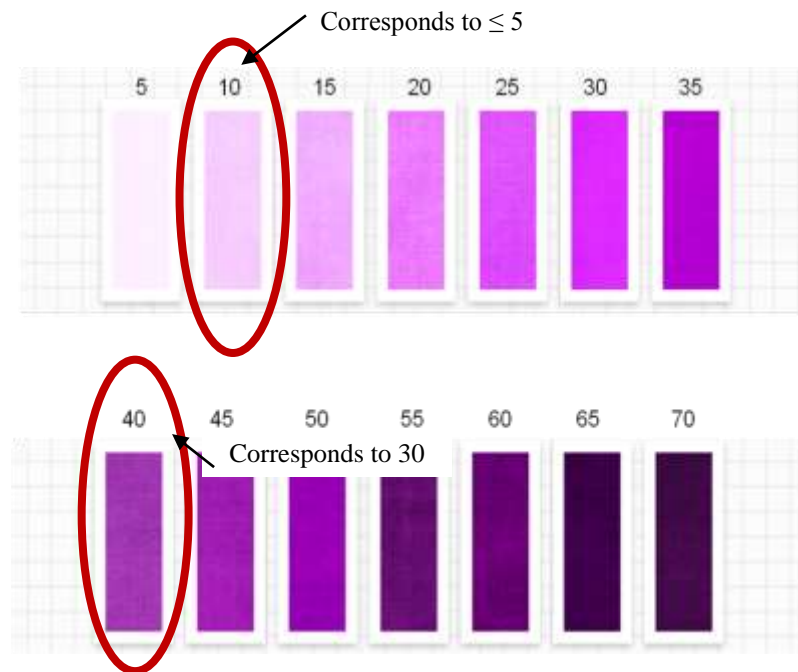


Figure 7. Respective Color Corresponding to ‘Poorly Iodized’ and ‘Adequately Iodized’ Salt Based on the Result of the Experiment

With clear distinction between levels of poorly and adequately iodized salts, three (3) corresponding charts representing either level or both levels of iodization were proposed and evaluated to check for its effectiveness. These different charts were proposed in the light of the existing RTK chart not matching the resulting color once applied by the solution. The 3 charts are as follows:

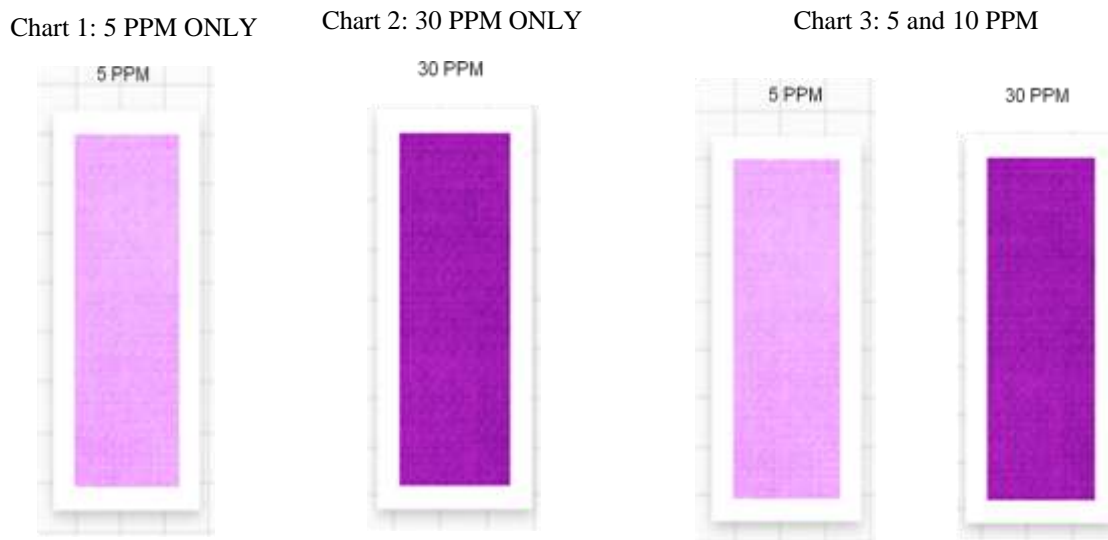


Figure 8. Three (3) Proposed Reference Color Charts for the Attributes Gage R&R Study

The evaluation was done through the conduct of Gauge Repeatability and Reproducibility (GR&R) Study using attribute measures. Attribute gage R&R (Repeatability and Reproducibility) is a tool to quantify how well the RTK measurement system is working. Four (4) iodine levels were utilized for the GR&R study, as follows:

- 1 Level for Non-iodized (≤ 5 ppm)
- 2 different Levels from Inadequately Iodized (10 and 20ppm)
- 1 Level from Adequately Iodized (30 ppm). Ideally, all 8 levels should be included.

With 4 levels of iodine concentration and 3 reference color charts, 12 data tables were generated and at 60 observations per table, total number of observations is 720 overall i.e. 360 observations per inspector. Two individuals were assigned as inspectors during the experiments.

Each batch of samples of 10 were given to the inspector 3 times, in new random order every time. The order of the batch and the order of samples in a batch were totally randomized using generated random numbers. As such, the inspectors were totally blind to the identity of the batch and the order of the samples in it.



Figure 9. Prepared Samples for Attribute Gage R&R Study (above); Same Same Samples After Application of Test Solution (below)

The following table presents the results of the Gage R&R conducted utilizing 2 inspectors and the 3 reference charts.

Table 4. Results of the Gage R&R Study Using Minitab’s Attribute Agreement Analysis

Attribute Agreement Analysis Results	Percentage Matched					
	Using Reference Chart with 2 Colors: 5 & 30 PPM		Using Reference Chart with 1 Color: 5PPM		Using Reference Chart with 1 Color: 30 PPM	
	Inspector 1	Inspector 2	Inspector 1	Inspector 2	Inspector 1	Inspector 2
Within Appraisers Appraiser agrees with him/herself across trials.	37.5%	55%	87.5%	95%	70%	37.5%
Each Appraiser vs Standard Appraiser's assessment across trials agrees with the known standard.	25%	37.5%	77.5%	85%	57.5%	25%
Between Appraisers All appraisers' assessments agree with each other.	25%		85%		22.5%	
All Appraisers vs Standard All appraisers' assessments agree with the known standard.	17.5%		77.5%		20%	

Based on the results, using the Reference Chart with the color for 5 PPM only yields the highest percentage with respect to the following four (4) measures: Within Appraisers, Each Appraiser vs Standard, Between Appraisers and All Appraisers vs Standard.

Within Appraisers (Inspectors)

Results showed that the percentage of agreement in Inspector’s 1 own individual assessments is 87.5%. For Inspector 2, the value is higher at 95%. The percentage refers to the inspector agreeing with himself across trials (Within Appraiser) and is called the individual *repeatability* of the inspector. This means that for Inspector 2 (at 95%), his judgements for the 3 trials matched in 38 out of the 40 units he inspected. Viewed differently, Inspector 2 is consistent with himself 95% of the time, using the Reference Chart with the color for 5 ppm.

The overall repeatability – the average of the individual repeatabilities – is 91.25%. This means that if the measurements are repeated on the same set of samples, the same results can be obtained 91.25% of the time.

Each Appraiser (Inspector) vs Standard (Reference Value)

Relative to the standard (i.e. the stated PPM of the unit observed), Inspector 1 matched the standard or reference value at least 77.5% of the time – at least in 31 out of the 40 units. For Inspector 2, it is 85%. The percentage refers to individual effectiveness i.e. the rate at which the inspector is in agreement with the standard or reference value.

Between Appraisers (Inspectors)

Interestingly, using the chart with only the reference color for 5PPM resulted in the two inspectors agreeing in their assessments 85% of the time i.e. their observations matched in 34 of the units observed. This percentage refers to the *reproducibility* of the measurement system. The 15% difference can be attributed to the differences in judgements between the inspectors with respect to some of the color changes that were shown to them. One inspector may have viewed a sample as poorly iodized while the other looked at it as being adequately iodized.

All Appraisers (Inspectors) vs Standard (Reference Value)

Of the 34 units where the two inspectors agreed in their judgements, 31 of those also matched the standard, for a 77.5% agreement among the inspectors and standard. The 77.5% is the overall effectiveness of the measurement system and is the percentage of the time the 2 inspectors agree and their agreement matches that of the stated standard for the salt samples.

Based on the figures obtained in Table 4, the following is a summary of the gage R&R measures for the three (3) reference charts

Table 5. Summary of the Gage R&R Measure for the Three (3) Reference Color Charts

Gage R&R Measures	Reference Charts		
	With 2 Colors: 5 & 30 PPM	With 1 Color: 5PPM	With 1 Color: 30 PPM
Overall Repeatability	46.25%	91.25%	53.75
Reproducibility	25%	85%	22.5%
Overall Effectiveness of the Measurement System	17.5%	77.5%	20%

Clearly, using the chart with 2 reference colors for 5 and 30 ppm is no better than using the chart with only 1 color for 30 ppm. If the measurements are repeated for the same set of samples, the use of either of these charts can generate the same results only 53.75% of the time, at the most. This is the same as saying that the results can go either way almost half of the time.

Furthermore, different inspectors using either of these charts will agree with each other only about 25% of the time. In 3 out of 4 times that they will both assess the same salt sample, after application of the test solution, their judgements will differ.

Finally, the overall effectiveness of the measurement system that utilizes either chart can only reach 20%, at best. That is, different operators evaluating the same salt sample will agree with each other --and with the standard-- in only 1 out of 5 times.

On the other hand, the measurement system using the Reference Chart with only the color for 5 ppm has shown that if measurements are done repeatedly on a particular sample, the same result will be obtained 91.25% of the time. Likewise, if different operators will perform the assessments, they will have the same judgements 85% of the time, and 77.5% of the time their matched results will agree with what the standard states.

The results of the Gage R&R study, especially on the measurement system using either the chart for 2 reference colors (5 and 30 ppm) and that of only the 30 ppm, validates the earlier conclusion obtained from the conduct of the experimental design regarding difficulty encountered by inspectors in distinguishing colors for inadequately iodized salt, relative to those for poorly iodized or adequately iodized salt.

On the use of the chart for 30 ppm only, any shade of purple for inadequately iodized salt that tends to be closer to the shade for adequately iodized is more likely be classified as the latter. This could result to more misclassification and a significant reduction in the overall effectiveness of the measurement system.

The same misclassification tendencies can also be observed with the use of the Reference Chart for 2 colors as shades closer to those of adequately iodized can be classified as such, even if it is not. From the experimental design, shades for inadequately iodized overlap more with those of adequately iodized than those with poorly iodized ones.

That said, inspectors tend to commit less misclassifications using only the Reference Chart for 5 ppm, a result confirmed by the gage R&R study.

Given a rule of thumb that anything above 90% for repeatability and reproducibility signifies an adequate measurement system, the measurement system using the chart for 5 ppm is close enough to be considered adequate [7]. The system can be fairly relied upon especially when the judgement is that the salt is poorly iodized. In cases however when the salt is not classified as 'poorly iodized', this measurement system has no means of determining whether it is inadequately or adequately iodized.

V. CONCLUSION

The study set out with its objective to evaluate the effectiveness of the Rapid Test Kit in determining the level of iodine content in the salt available in the market, whether it belongs to the adequately iodized salt (30 ppm and above), non-iodized (5 ppm and below) and inadequately iodized salt (above 5 ppm and below 30 ppm) classification.

As far as instructions contained in the RTK regarding the application of the test are concerned, the study found that they are easy to follow and no complicated training is required in its administration.

A major concern lies in the color chart included in the kit. The colors in the chart simply did not match those observed when test solutions were applied on the samples. The tests resulted to colors representing different shades of purple while the existing color presents shades of gray instead.

It was shown by the experimental design (RCBD Model) that inspectors can clearly distinguish between samples containing iodine concentration of 5 ppm (poorly iodized) and ≥ 30 ppm (adequately iodized) based on the resulting shades. On the other hand, inspectors had difficulty assessing samples having levels of iodine concentration within the inadequately iodized range. Iodine concentration levels at the lower rung of this range (e.g. 10 ppm) tend to be misclassified as 'poorly iodized' while those at the upper end of the range (e.g. 20, 25 ppm) tend to be judged as 'adequately iodized'. Interestingly, the result also showed that a salt sample with 15 ppm concentration can be misclassified by inspectors either way.

With these findings, the use of the RTK to classify the tested salt on the three (3) levels of iodization can be problematic. The results of the Attribute Gage R&R confirmed this finding. Of the three (3) proposed Reference Charts to replace the existing chart, the one involving one color only for 5 ppm was the most effective i.e. high percentage of agreement between inspectors on their judgements and that their judgements match the actual iodine levels. The very low Reproducibility and the Overall Effectiveness of the measurement system involving the two other reference charts (5 & 30 ppm and 30 ppm only) can be attributed to these misclassifications of inadequately iodized salt as adequately iodized.

If the government will continue to use the RTK measurement system, the reference chart for one color for 5 ppm is the preferred replacement. However, its limitation should be recognized in that it can only confidently state that a salt sample is either 'poorly iodized' or *at the very least*, inadequately iodized.

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