Market-level Rapid Assessment of Fortified Foods In Indonesia, Kenya, and India

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EXECUTIVE SUMMARY

The market-level rapid assessment has been completed in three countries: Indonesia, Kenya and India. The objectives of this project were to:

- establish a rapid testing methodology that can be employed at affordable time & cost investment and produce reliable results of micronutrient (MN) levels in fortified food items.
- baseline measurement of current fortification levels in the target geographies to inform local stakeholders of the quality and program progress.

Nutrition International (NI) coordinated the project between QuImpact and local stakeholders in each country. QuImpact was commissioned by NI to consult on sampling protocol, organize the training of local analysts, the testing of samples and the data analysis. The target vehicles and parameters were:

- Indonesia: Vitamin A in edible oil
- Kenya: vitamin A and iron in wheat and maize flour
- India: vitamin A in edible oil and iron in fortified rice kernels (FRK).

In Indonesia sampling was performed by KFI specifically for this project. Only 30% of the total edible oil that is available in packaged form falls under mandatory fortification regulation in Indonesia. 84% of collected samples from 9 major brands that cover over 85% of this segment were fortified with Vitamin A according to national standard (>45 IU RE/g). This corroborates the figures of 90% as reported by BPOM, the national regulatory body.

In Kenya samples were obtained from TechnoServe, who collected them for a separate study. A total of 87 wheat flour samples covering ~80% of the wheat flour sector, and 39 maize flour samples covering approximately ~40% were tested. 22% of wheat and 5% of maize samples had vitamin A content within the range specified in the national standard (0.5-1.4 mg/kg). 86% of wheat and 62% of maize samples had iron content according to national standard, >= 20 & 21 mg/kg, for wheat and maize, respectively. *The results are to be compared to the observations made by MOH/JKUAT, TechnoServe and the Medalion laboratory.*

In India, fortified oil samples were obtained from GAIN, who collected them for a separate study. Here, samples were collected from two states: Tamil Nadu and Madhya Pradesh. 28% samples had vitamin A content within the national standard range (6-9.9 µg RE/g), while 43% vitamin A content were below the limit of quantification (LOQ) of 3.0 µg RE/g (µg RE/g = mg RE/kg). *The results are to be compared to the observations made by GAIN and the other laboratory in India.*

The 26 FRK samples were collected by PATH directly from FRK manufacturers in India. For this vehicle, 62% tested for iron content within the national standard of (2800-4250 mg/kg). In total, 25 samples had iron level above iCheck Iron's limit of quantification (LOQ) 787 mg/kg.

The analytical methods used in this study for the qualitative assessment of iron in flour and FRK, as well vitamin A detection in oil and flour are explained in each country section. Qualitative tests for iron are effective at detecting fortified samples, qualitative test for vitamin A in oil when concentration is above 33 IU/g is also effective at detecting vitamin A fortification. Rapid quantitative testing with iChecks is also an efficient approach to generate quantitative assessment on the proportion of the samples in line with national standards. The results with iChecks are comparable to traditional laboratory methods (AAS, HPLC, ICP) performed at accredited labs locally as well as in Germany.

			Qualitative Test		Compared to national standard:		
		Number of Samples	YES	NO	Fortified according to national standard	Below national standard	Above national standard
INDONESIA	Edible Oil – Vitamin A						
	Qualitative	479	99%	1%	-	-	-
	iCheck Chroma 3	479	99%	1%	85%	15%	
	Local accredited Laboratory (HPLC)	100	98%	2%	60%	40%	-
	External accredited Laboratory (HPLC)	100	98%	1%	84%	16%	-
KENYA	Wheat and maize flour – Vitamin A						
	Qualitative - wheat	87	28%	72%	-	-	-
	Qualitative - maize	39	13%	87%	-	-	-
	iCheck Fluoro - wheat	87	48%	52%	22%	74%	4%
	iCheck Fluoro – maize	39	41%	59%	5%	92%	3%
	Local accredited Laboratory (HPLC)	32	75%	25%	44%	47%	9%
	External accredited Laboratory (HPLC)	32	38%	63%	16%	84%	-
	Wheat and maize flour – Iron						
	Qualitative - wheat	81	81%	19%	-	-	-
	Qualitative - maize	39	68%	32%	-	-	-
	iCheck Iron - wheat	81	97%	3%	86%	14%	-
	iCheck Iron - maize	39	83%	18%	62%	38%	
	Local accredited Laboratory (AAS)	32	79%	21%	65%	35%	-
	External accredited Laboratory (ICP/MS)	32	78%	22%	75%	25%	-
INDIA	Edible Oil – Vitamin A						
	Qualitative	103	43%	57%	-	-	-
	iCheck Chroma 3	103	43%	57%	28%	68%	4%
	Local accredited Laboratory (HPLC)	28	79%	21%	21%	79%	-
	External accredited Laboratory (HPLC)	28	46%	54%	7%	93%	-
	FRK – Iron						
	Qualitative	26	92%	8%	-	-	-
	iCheck Iron	26	97%	3%	62%	38%	-
	Local accredited Laboratory (AAS)	26	96%	4%	50%	50%	-
	External accredited Laboratory (ICP/MS)	26	96%	4%	65%	27%	2%

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CONTENTS

RECOMMENDATIONS FOR EXECUTING RAPID ASSESSMENT

The following are the recommendations and considerations when planning and executing rapid assessment of fortified foods quality in the markets.

1. **PLANNING**

a. Engage Local Stakeholders:

- Early Involvement: Involve local stakeholders (i.e. NGO partners, academia, monitoring authorities) early in the planning phase before setting budgets and timelines. Their support and input are essential for smooth project execution.
- Consensus Building: Conduct meetings and workshops to build consensus on project goals, methodology, and expected outcomes. Stakeholders buy-in is vital for successful implementation.
- Focus: Only engage the stakeholders that are required to have the approvals or resources to conduct the assessment. Too many different interested parties will make the project costly and complicated.

b. Sampling Strategy:

- Dedicated Sampling: Plan for dedicated sampling immediately before testing to prevent degradation of micronutrients, ensuring the reliability of the samples.
- Target Coverage: Develop a sampling strategy that meets your requirements (i.e target geography, brands covering 80% of the market, target retail type).

c. Resource Allocation:

- Timely Delivery of Resources: Ensure the timely procurement of all necessary materials, equipment, and reagents. Consider the additional time needed for resources to be sourced from outside the country.
- Allocate personnel: recruit the personnel required for the scale and scope of sampling, testing, and data analysis.
- Training Programs: Assess and conduct necessary training sessions for personnel to ensure they are well-prepared and capable of executing their tasks effectively.
- Control Samples: Ensure the availability of adequate control samples to validate testing methods and results.

2. **EXECUTION**

a. Follow SOPs: Develop detailed Standard Operating Procedures (SOPs) for each step of the sampling and testing process. Train personnel to adhere strictly to these procedures to ensure consistency and accuracy.

b. Sample Handling:

- Secure Packaging: Carefully package and label samples to prevent contamination or degradation. Consider potential leakages, exposure to UV light if using transparent packaging, and ensure the sample ID/label is securely attached.

- Effective Tracking: Implement robust tracking systems for sample IDs, preparation data, results, and timestamps. Utilize digital tools for enhanced efficiency and accuracy.

c. Method Verification:

- Spiked Control Samples: Verify and optimize testing protocols using spiked samples before testing actual market samples. This ensures the reliability and consistency of results for both rapid methods and reference laboratories.
- Comprehensive Documentation: Maintain detailed records of all standards, spiked samples, control samples, and duplicates to facilitate accurate data analysis.

d. Data Collection:

- Visual Documentation: Take photos and videos during sampling and testing to document the process and provide visual references.
- Meticulous Logging: Record all data meticulously, including sample IDs, testing results, and timestamps.

3. ANALYSIS AND REPORTING

a. Data Analysis:

- Clear Criteria: Define the required data sets and methods for analyzing them. Establish criteria for interpreting fortified, non-fortified, and adequately fortified samples.
- Comparative Analysis: Compare results against national standards and interpret findings in the context of program goals, such as average fortification levels, differences between brands, geographies, or differences between sales locations (e.g., open markets vs. supermarkets).

b. Reporting:

- Standardized Templates: Use pre-developed templates for data analysis and reporting to ensure consistency and speed up the process.
- Effective Communication: Prepare comprehensive reports that clearly communicate findings to stakeholders. Include actionable recommendations for improving fortification programs based on the results.

4. CONTINUOUS IMPROVEMENT

a. Feedback Mechanism: Establish a feedback loop to gather insights and suggestions from stakeholders and field personnel. Use this feedback to refine methodologies and improve future assessments.

b. Ongoing Training: Invest in ongoing training and capacity-building initiatives to ensure that local analysts and stakeholders are well-equipped to carry out rapid assessments independently.

CHOOSING TEACHING METHODS

Qualitative test iron test:

- Qualitative tests (i.e. iron spot test with Na Thiocyanate, HCl and hydrogen peroxide) are effective for detecting the presence or absence of added iron in wheat and maize flour.
- The same test is effective at detecting iron in FRK; and presence of FRK in normal rice.
- It is also effective in differentiating ferric from ferrous iron.

Qualitative test vitamin A test:

- Qualitative test (i.e. colorimetric vitamin A test with TFA/DCM) is not recommended for low concentration of vitamin A in wheat and maize flour due to complex handling and subjective interpretation.
- Qualitative test (i.e. colorimetric ring test with chloroform and antimony trichloride) is also not recommended for low concentration of vitamin A in edible oil due to hazardous chemicals and subjective interpretation.
- The qualitative test (i.e. colorimetric test with TCA/DCM) is effective at detecting vitamin A presence in edible oil above 33 IU/g.

Rapid methods – quantitative testing of iron with iCheck Iron:

- iCheck Iron is effective in testing the quantity of iron in the fortified wheat flour when analysts are well trained with spiked samples and the appropriate sample preparation protocol is applied (i.e. 0.2MHCl for ferrous fumarate; or HCl & NaOH for FRK).
- The results with iCheck Iron correlate quite well with results obtained by accredited laboratories with AAS/ICP: Pearson of 0.76 to 0.80. Also there is strong alignment in the portion of samples classified as within national standard and outside using iCheck and ICP/AAS.

Rapid methods – quantitative testing of vitamin A with iCheck Chroma 3:

- iCheck Chroma 3 is effective in testing the quantity of vitamin A in the fortified edible oil when analysts are well trained with spiked samples and when the measurement range of iCheck Chroma 3 (10 to 100 IU/g) is fit for purpose.
- The results with iCheck Chroma 3 correlate well with results obtained by accredited laboratories with HPLC: Pearson of 0.86 to 0.91.

Rapid methods – quantitative testing of vitamin A with iCheck Fluoro:

- iCheck Fluoro is effective in measuring added vitamin A in wheat and maize flour. However, analyst training is critical as well as understanding matrix effect and how to troubleshoot.
- The preparation of reliable and stable control samples is a significant challenge. The number of control samples analyzed by reference methods at accredited laboratories (i.e. HPLC) is not sufficient to draw conclusions on the correlation. However, there is a strong alignment in the portion of samples classified as within national standard and outside using iCheck and HPLC at the German accredited laboratory.

Laboratory methods at accredited laboratories

- Despite being accredited, it is strongly recommended to regularly send in multiples of spiked control samples with the market samples to those labs. This will provide the assessment of recovery and variation necessary for adequate interpretation of results.
- Variation is inherent for testing low levels of micronutrients in fortified foods also with accredited laboratories and should be assessed and integrated into interpretation.
- It is recommended to send in at least 3 different concentrations in blinded triplicates.

CHALLENGES AND CONSIDERATIONS

Resource Constraints: Fortification program officers may face challenges related to limited resources, including budget constraints and the availability of trained personnel. Adequate planning and resource allocation are essential to overcome these challenges.

Logistical Issues: Logistical challenges, such as delays in the procurement of materials and equipment, can disrupt the project timeline. Ensure timely availability of all necessary resources from local suppliers.

Importation Problems: Importation of equipment and reagents like iChecks can cause delays due to customs regulations. It is crucial to engage early with the distributors of BioAnalyt, manufacturers of equipment, to get the process started.

Data Accuracy: Ensuring data accuracy is paramount. Careful adherence to SOPs, proper sample handling, and comprehensive documentation are necessary to prevent errors and ensure reliable results.

Stakeholder Coordination: Coordinating with multiple stakeholders can be complex and time-consuming. Effective communication and consensus-building strategies are essential to ensure stakeholder support and collaboration.

Technical Challenges: Interpreting results for samples with low micronutrient concentrations can be challenging. Extra care must be taken in such cases to ensure accurate interpretation and avoid discrepancies.

Implementing rapid assessment studies for fortification levels is a critical step for government agencies and NGOs to monitor and enhance food fortification programs. While there are challenges to be aware of, a well-planned and executed assessment can provide valuable insights and drive improvements in public health nutrition. By following the outlined steps and addressing potential difficulties proactively, government agencies and NGOs can ensure the success of rapid assessment studies and contribute to the overall effectiveness of fortification initiatives.

QuImpact's learnings throughout this project is extensive and practical, and we are keen to share the learnings, keep building up local testing capacity and continuously support monitoring efforts.

BACKGROUND

The BMGF Nutrition team's overarching goal is to catalyze reliable, self-sustaining LSFF systems to deliver micronutrients to the most in need and ensure all women and children have the nutrition they need to live healthy and productive lives. A key part of the reliability and sustainability of a system is through compliance monitoring of fortified foods to ensure that the micronutrient levels in the food adequately meet the country-specific policy and regulatory standards. However, compliance systems have been a weak point in most fortification programs to date, frequently due to poor government monitoring and enforcement with limited resources, especially constrained budgets.

Rapid testing of micronutrients in fortified foods has the potential to provide faster insight into the compliance levels at lower costs and minimal human resources. The project's initial objective was to investigate the usability of a rapid testing device and survey methodology across multiple geographies to inform whether large-scale food fortification (LSFF) is sustained across the relevant coverage area, which is the core of BMGF Nutrition Strategy. The project focused on India - where there are active fortification projects on oil and rice, Kenya - where fortified flour has been a main focus for improving compliance, and Indonesia - where a local leading partner in LSFF is being strengthened and there is an identified need for measuring the marketplace.

In early 2023, a rapid testing approach was employed in Nigeria by BMGF to test foods mandated for fortification for adequate micronutrient (MN) levels. The testing approach utilized both qualitative "yes/no" rapid testing kits and quantitative rapid testing devices (iCheck devices from BioAnalyt https://www.bioanalyt.com/). The results from this testing were comparable to the MN levels found with laboratory testing from previous studies. With a faster turnaround of results for a fraction of the costs with laboratory testing, this methodology has strong potential to enable improved monitoring practices for fortification programs.

To further validate and standardize this rapid market assessment methodology a project was designed by QuImpact gGmbH and executed with support from Nutrition International and BMGF to repeat rapid assessment in three other settings. Namely:

- India: Vitamin A in edible oil; Iron in Fortified Rice Kernels (FRK)
- Kenya: Vitamin A and Iron in flour
- Indonesia: Vitamin A in edible oil

The key intended outcomes were:

- Established rapid testing methodology that can be employed with affordable time/cost investment and produces reliable results of MN levels in fortified food.
- Baseline measurement of current fortification levels in the target geographies to inform local stakeholders.

The testing approach was targeted to follow the below framework in each geography:

- Representative sampling of the fortified food vehicle in the target regions (to be informed by currently available market analyses) by a local implementation partner
- Testing of 500 food samples per food vehicle with qualitative yes/no test kits to assess presence/absence of MN fortification
- Subsequent testing of food samples using iCheck device for quantitative MN measurement
- Analysis of the data against the local regulations and standards to assess level of compliance
- Data analysis and final report of results
- To build further trust (of the device, and for government stakeholders' buy-in 20% of the samples to be tested by accredited, conventional laboratory (i.e. HPLC, ICP, AAS).

This document summarizes the actual process that took place in the three target countries, observations, analysis of data and learnings with a proposal how to plan and implement rapid market-level assessment of fortified foods as regular, cost-effective and efficient methodology.

By implementing this structured approach, the project aims to provide a rapid yet thorough assessment of food fortification quality. The findings will not only help in verifying current fortification practices but also guide future policies and interventions to enhance the nutritional quality of food products in target countries This initiative is a significant step towards mitigating micronutrient deficiencies and promoting better health outcomes.

METHODOLOGY

To ensure comprehensive and reliable results, the project has following framework in each country:

Sampling: This sampling was informed by currently available market analyses and carried out by local implementation partners. The aim was to capture a broad and accurate picture of the fortification landscape. A maximum of 500 samples per fortified food vehicle were collected and subjected to MN testing.

Testing: All samples were tested where relevant and possible with qualitative and quantitative methods.

i. Qualitative methods to assess whether the target MN is present or not in the sample.

ii. Rapid quantitative methods, iCheck, to quantify the level of MN in all samples of each food vehicle.
iii. Quantitative method at accredited laboratory to quantify the level of MN in min 20% of samples from each food vehicle.

Result analysis: The results for each method were compared by the proportion of samples that have MN levels within the range provided in the national standard level or outside. The methods were further assessed where possible for recovery, precision, false positives or false negatives.

Learnings and recommendations: for each country the observations were noted down in terms of what it meant to organize the stakeholders, keep the time frames, ability to follow intended methodology, cost of analysis. The learnings and recommendations are noted down how to plan and execute such assessment on routine basis in different settings.

PROJECT TIMELINE

The Figure 1 illustrates a high-level chronogram of a project across three countries: Indonesia, Kenya, and India. The project spans from November 2023 to May 2024, highlighting key activities such as kick-off meetings, sample collection, sample testing, and reporting.

Coordination and logistics took most of the time in this project. The analysis with iChecks took the shortest time. If there is a dedicated sampling and testing team (without parallel assignments) to perform rapid assessment the overall project can be completed within 3 months period.

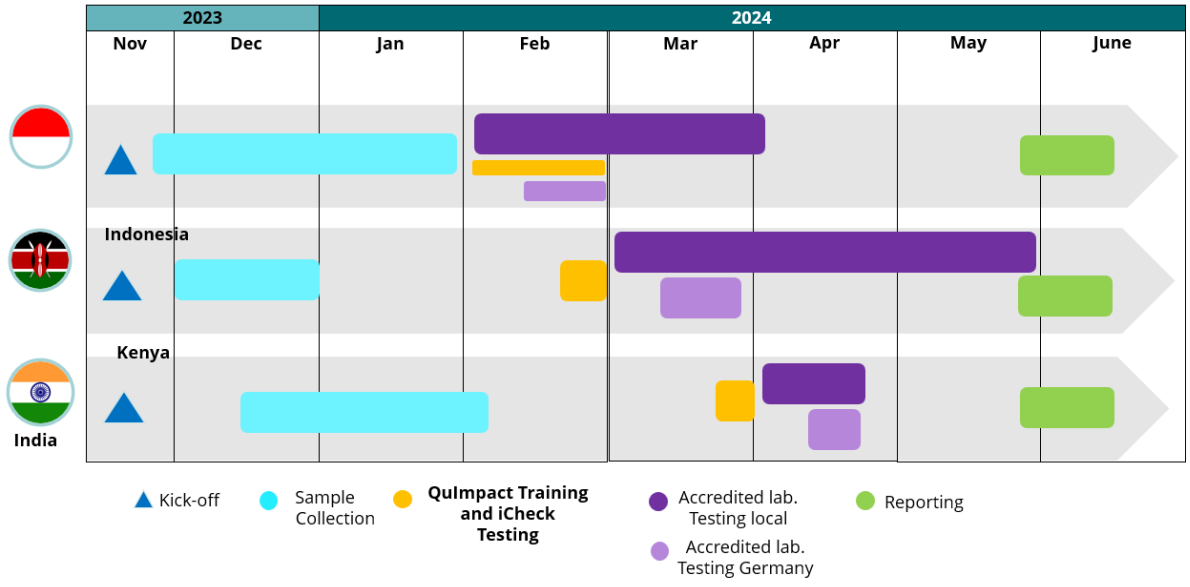

Figure 1: Timeline of Rapid Assessment of Food Fortification Project Across Indonesia, Kenya, and India.

PROJECT IMPLEMENTATION

1. INDONESIA

BACKGROUND

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Fortification of cooking edible oils with Vitamin A in Indonesia is currently part of the National Strategy for Stunting. This initiative aims to prevent the significant impact of Vitamin A deficiency in vulnerable segments of the population, particularly young children and women of reproductive age. Vitamin A deficiency in these groups can lead to preventable blindness and increased susceptibility to infections.

In Indonesia, fortification is divided into two categories: mandatory and voluntary. Mandatory fortification is part of the government's nutrition improvement program, while voluntary fortification is carried out by private enterprises, which are only regulated for food security requirements. Currently, several types of food are subject to mandatory fortification, including salt fortified with iodine, cooking oil with vitamin A, and wheat flour with iron, zinc, thiamin, riboflavin and folic acid. With the obligation to fortify palm cooking oil and flour, it is feasible that all families can fulfill their nutritional intake, contributing to overall stunting prevention efforts.

A study conducted in West Java demonstrated that fortified cooking oil contributed significantly to the daily recommended intake of vitamin A, particularly benefiting children and women by improving their serum and breast milk retinol levels (Martianto et al., 2011). Fortifying cooking oil with vitamin A has proven to be an effective strategy to improve vitamin A intake and overall nutritional status in these vulnerable groups in the last couple of years.

The palm oil refining industry in Indonesia is consolidated, with about 77 production companies, of which the top 5 supply 70% of domestic requirements (UNICEF, 2023). The 77 production companies have around 300 refineries, with the greatest number in West Java, Central Java, and East Java. Other important producing areas are North Sumatra and DKI Jakarta. Traditionally, most domestic oil for food consumption has been sold in bulk, either to food producers or retail markets, allowing households to purchase unbranded oil in small, affordable amounts.

Indonesia's low-income groups generally consume unbranded vegetable oil, with an average consumption of approximately 25 grams/day. By 2012, unbranded oil constituted approximately 70% of the total oil traded in the country (Soekirman, 2012). In 2022, the government launched the "People's Cooking Oil Programme," aiming to provide fair and equal access to affordable cooking oil for the public. The program includes requiring oil refineries to fulfill domestic market obligations before they can get export quotas, establishing a maximum retail price, and requiring oil to be packaged under the Minyakita brand. The program appears to have been successful in increasing the proportion of household cooking oil that is packaged. BPOM reports that there are 157 registered producers and packagers of Minyakita in 16 provinces and 485 registered Minyakita brands for the years 2022 and 2023 (UNICEF, 2023).

Since 2019, the Indonesian National Standard (SNI) 7709:2019 has required that all palm cooking oil contain 13.5 mg/kg or 45 IU/g of vitamin A. This regulation is enforced under the Ministry of Industry Regulation No. 46/2019, which mandates that all producers, packers, and importers comply with these fortification standards. The enforcement of this standard became fully effective on February 1, 2023, after several postponements to allow the industry to adapt. The SNI for palm cooking oil was updated in 2019 to allow the required vitamin A content to be made up of both synthetic vitamin A – retinol palmitate – and pro-vitamin A or beta carotene, calculated as the vitamin A equivalent.

Regulatory monitoring for cooking oil fortification is primarily undertaken by the National Agency of Drug and Food Control (BPOM) at both production and market levels. BPOM has registered 297 production and packaging facilities for cooking oil. BPOM market surveillance data indicates that more than 90% of samples tested were compliant with fortification requirements between 2016 and 2023. Importantly, such market surveillance is focused on packaged products in the marketplace, not including cooking oil sold in bulk for repackaging.

Mandatory fortification in Indonesia currently applies only to packaged cooking oil, while bulk cooking oil, which constitutes a significant portion of the market (around 70% of total consumption), is not yet fully covered by these regulations (KF Indonesia, 2023).

Despite these efforts, challenges remain. Vitamin A is unstable during storage, especially in packaging exposed to oxygen and sunlight, which can decrease vitamin A levels. The continued commitment of the Indonesian government and industry stakeholders to fortification efforts is crucial for addressing vitamin A deficiency and improving public health outcomes in Indonesia. In this context, it is crucial to develop tools that enhance and streamline market surveillance. These efforts are most effective when supported by local legislation that backs fortification initiatives and facilitates effective interventions.

SAMPLE COLLECTION

NI supported project preparation and stakeholder coordination with the Indonesian Food Fortification Coalition (KFI). The sampling collection and procurement processes were executed by KFI personnel, following a sampling protocol developed in coordination with NI and QuImpact (see Annex 1).

Palm cooking oil samples for the survey were collected from Jakarta and Surabaya, with Jakarta accounting for 79% (n=377) of the samples, while Surabaya represented 21% of the samples (n=102), reflecting their respective population distributions. In Jakarta, samples were procured from five locations: South Jakarta, East Jakarta, Central Jakarta, West Jakarta, and North Jakarta. In Surabaya, samples were collected from various locations within Surabaya City. Samples were gathered from each brand in volumes of 0.5 L, 1 L, or 2 L, from large retail stores, minimarkets, and traditional markets. A total of 479 packs of palm cooking oil were collected for testing.

This study employed a survey design to assess the Vitamin A content of palm cooking oil from top brands, which account for an 85% market share, and government-subsidized palm cooking oil, which holds a 15% market share, collected from five areas of Jakarta and Surabaya City. This approach ensured a representative sample, providing robust data to accurately evaluate Vitamin A levels in palm cooking oil.

TRAINING METHODOLOGY

One of the most important steps prior to the implementation phase of the project was to provide training to local analysts in testing methodologies. During the on-site phase, it was expected that, following the training, the newly trained analysts would be able to perform testing of all samples with minimal supervision. To this end, two BioAnalyt instructors (Dr. Santiago Andrade, Dr. Anna Zhenchuk) were aiming to train up to three key analysts from the host institution, KFI in Jakarta.

At the beginning of the session, the trainers provided a comprehensive guide on sample handling procedures and described the protocols for qualitative colorimetric testing, including the preparation of the necessary solutions. Following this, an in-depth introduction to the iCheck Chroma 3 was given, covering step-by-step calibration of the device, sample preparation, readout, and interpretation. Results of the training session in Indonesia are displayed in Annexes. Furthermore, detailed instructions for each technique are outlined in the following sections.

TESTING METHODOLOGY

In Indonesia, the testing methodology for the analysis of vitamin A in edible oil samples began with qualitative colorimetric test. This colorimetric test allowed analysts to provide a preliminary assessment of the presence of vitamin A in the samples. The readout of this

experiment resulted in a Yes (fortified) /No (not fortified) decision based on the visual evaluation of the reaction.

Subsequently, a quantitative analysis using the iCheck Chroma3 device was conducted to obtain precise measurements of the vitamin A content. To ensure quality control, spiked control samples of edible oil were analyzed after every tenth sample, and every tenth sample was analyzed in duplicates using the same protocol.

At the end of this workflow, approximately 20% of the analyzed samples were sub aliquoted for further verification using reference methodologies in accredited laboratories. Specifically, BioAnalyt/QuImpact trainers took 100 samples for testing with HPLC at an external laboratory in Germany. It is important to note that the samples chosen for analysis in Germany were not the same as those provided by KFI to the local reference laboratory, since samples for HPLC analysis at the local reference laboratory were sent in advance to BioAnalyt/QuImpact trainer's arrival to Jakarta.

QUALITATIVE TESTING: Colorimetric Assay

Colorimetric qualitative analysis is used to screen for the presence of vitamin A in samples. This protocol is an adaptation of BASF - Method of analysis AM/00917/01e - "Semi quantitative colorimetric determination of Vitamin A Palmitate in fortified sunflower oil" This method involves introducing a chromogenic reagent to a vitamin A fortified oil sample, which reacts with retinol to produce a distinctive but transient blue color complex. The intensity of the color is directly proportional to the concentration of retinol, allowing the analyst to visually identify the presence of Vitamin A in the sample. The protocol was adopted to decrease the reagents volume per sample (80% decrease for ascorbic acid solution; 33% decrease for TCA/DCM solution). Below are the instructions for the preparation of the solutions and the sample analysis:

Solution Preparation

- Solution 1: Prepare a supersaturated solution with TCA (Trichloroacetic acid) in DCM (Dichloromethane). TCA is the chromogenic reagent that reacts with retinol and forms a blue color complex.
- Solution 2: Prepare 25% ascorbic acid in distilled or bottled water. This solution ensures that the retinol remains in a reduced state, thereby improving the accuracy of the colorimetric measurement.

To prepare the above solutions, the following table provides a reference based on the number of samples to be analyzed, along with the corresponding calculated dilutions for the chemical reagents.

Table 1: Dilution chart for solution preparation to prepare a TCA/DCM based qualitative colorimetric testing.

	Chemical	*Scaling factor*	Number of Sample			Units
			500	**100**	**10**	
Solution 1	**TCA**	**1.15**	575.0	115.0	28.8	g
	DCM	**0.57**	283.0	56.6	14.2	mL
Solution 2	**Ascorbic acid**	**0.02**	8.3	1.7	0.4	g
	Water	**0.07**	33.3	6.7	1.7	mL

The use and handling of these chemicals require protective gloves and goggles to prevent severe skin irritation. These solutions remain stable for approx. two weeks when refrigerated.

Sample Analysis

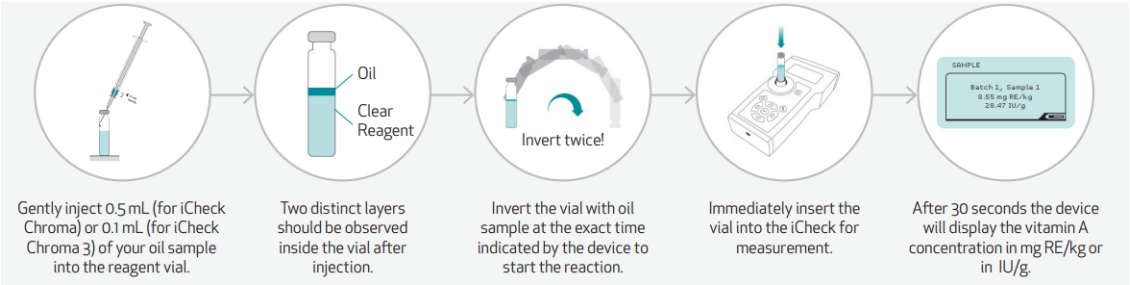
- Step 1: Measure in 0.5 mL oil sample with a pipette or a syringe into a transparent container (minimum 3 mL volume). Add 0.2 mL ascorbic acid solution (Solution 2). Shake for 2 minutes.
- Step 2: Add 2 mL TCA solution (Solution 1) to 0.7 mL of oil and ascorbic acid mix from STEP 1.
- Step 3: Observe color change within 5 seconds. If blue color appears then it is positive for vitamin A, if no blue color it is negative for vitamin A presence in the edible oil.

QUANTITATIVE TESTING: iCheck Chroma 3

The iCheck Chroma 3 is used for the determination of vitamin A in edible oil. This method is based on the Carr-Price reaction, where the reagents in the vial turn a brilliant blue in response to retinol, with the intensity proportional to the retinol concentration. The

fundamental principle involves the reaction of retinol with antimony trichloride ($SbCl_3$) to generate a transient blue color. The iCheck Chroma 3 measures the absorption of this color at three different wavelengths over 30 seconds, calculating the vitamin A content through a sophisticated algorithm and displaying the result in mg retinol equivalents (RE) /kg of oil. The device has a linear range of 3–30 mg RE/kg. Considering that the national standard in Indonesia is 45 IU/g of vitamin A (total), meaning the is the sum of Vitamin A and pro vitamin A (carotene) calculated as the equivalent of Vitamin A, this is equivalent to 13.5 mg RE/kg. Therefore, in principle, the expected fortification value in our samples should not require any dilution and can be tested directly.

The following is the sample preparation and analysis workflow for Vitamin A measurement:



A description of how to measure the sample is shown in the following training video: https://www.youtube.com/watch?v=s2Kyg90qyz0.

This method has been validated against reference methods in several publications. Most recently, a study compared a portable device to high-performance liquid chromatography (HPLC) in terms of quantification of vitamin A in both spiked and commercially fortified oils, taking measurements of nine different oil types (soybean, palm, cottonseed, rapeseed, corn, peanut, coconut, sunflower, and rice bran) spiked with retinyl palmitate at six different concentrations. Vitamin A recoveries were 97–132% for HPLC and 74–127% for iCheck Chroma 3, including a strong positive correlation, $r = 0.88$. Concluding that iCheck provides a lower-cost, quick, and user-friendly alternative to HPLC with comparable performance (Palma Duran et. al, Food Anal. Methods, 2024).

RESULTS

In total, all 479 collected samples were tested for qualitative vitamin A testing. From these samples, 99% (n=475) tested positive for fortification, while only 0.84% (n=4) did not show evidence of fortification.

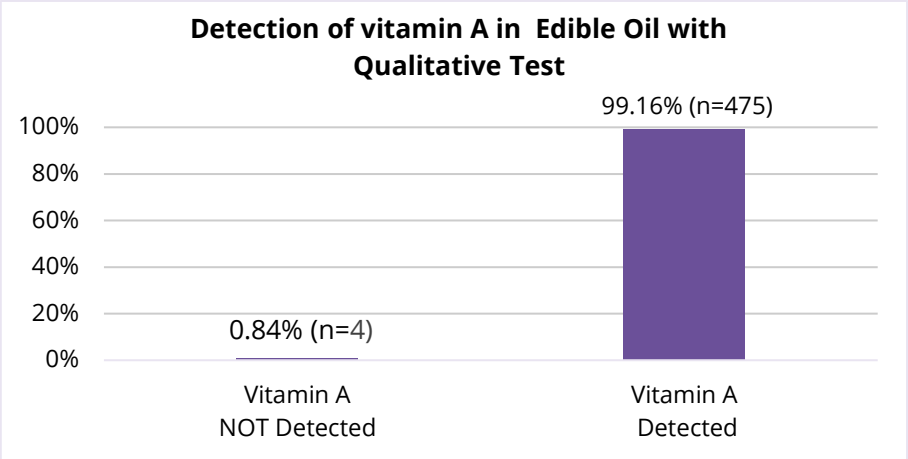


Figure 2: Qualitative assessment of vitamin A in oil with quantitative test (n=479).

Indonesia has a national standard for fortification with Vitamin A in edible oil of >= 45 IU/g (13.5 mg RE/kg). Considering that iCheck Chroma 3 linear range is 3-30 mg RE/kg or 10-100 IU/g. Results with iCheck were grouped in the following way:

- Below linear range <10 IU/g
- Below national standard 10 – <45 IU/g
- According to national standard >= 45 IU/g

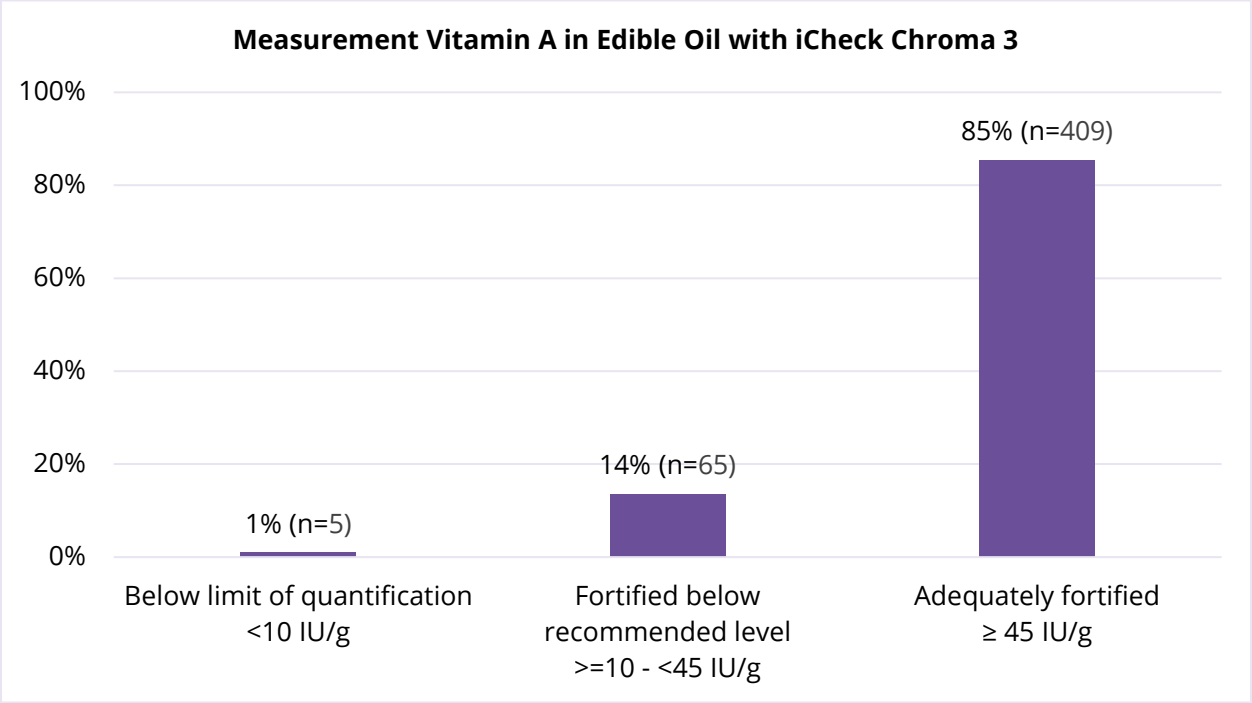


Figure 3: Measurement Vitamin A in Edible Oil with iCheck Chroma 3 (n=479).

The results from the iCheck Chroma 3 indicate that a majority of edible oil samples (85%) are adequately fortified with Vitamin A. Additionally, 14% of the samples were fortified but below the recommended level, containing between 10 IU/g and 45 IU/g. A minimal number of samples (1%) had Vitamin A levels below the detection limit of 10 IU/g (lower detection limit of iCheck Chroma 3 is 3.0 mg RE/kg). Overall, the data supports the feasibility and effectiveness of using rapid testing devices for market-level assessment of fortified foods.

The comparison between the iCheck Chroma 3 and qualitative vitamin A test results, as further analyzed below, indicates a high level of agreement for results obtained from qualitative Vitamin tested samples that detected Vitamin and iCheck Chroma 3A. Importantly, measurement of vitamin A concentrations with iCheck increases the resolution for this analysis, since it enables us to discriminate between groups of presence of vitamin A. In this sense, adequately fortified samples completed 86% (n=409) of positive samples, meanwhile, samples fortified below the recommended national standard completed 13% (n=64). Those are samples that, in principle, tested positive for presence of vitamin A, however, further analysis unraveled that the level of vitamin A was below the national standard requirement. It is possible that vitamin A is present, but very low levels.

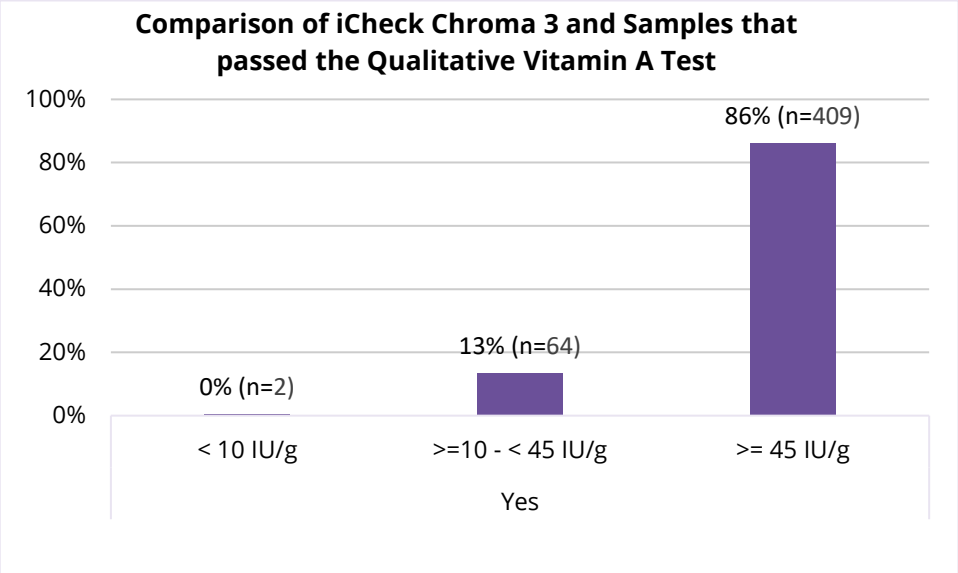


Figure 4: Comparison of iCheck Chroma 3 and qualitative vitamin A test results in edible oil samples. Samples that passed the qualitative vitamin A test (Yes, n=475).

Contrarily, minor discrepancies between qualitative and quantitative iCheck results were observed across samples that did not pass the qualitative test. In this sense, for those samples where No-vitamin A was detected (n=4), only one sample generated a measured value when testing with iCheck Chroma 3. For all the rest of the samples, iCheck confirmed the no presence of vitamin A. Overall, the total number of samples measured with qualitative vitamin A test and iCheck Chroma 3 were 479.

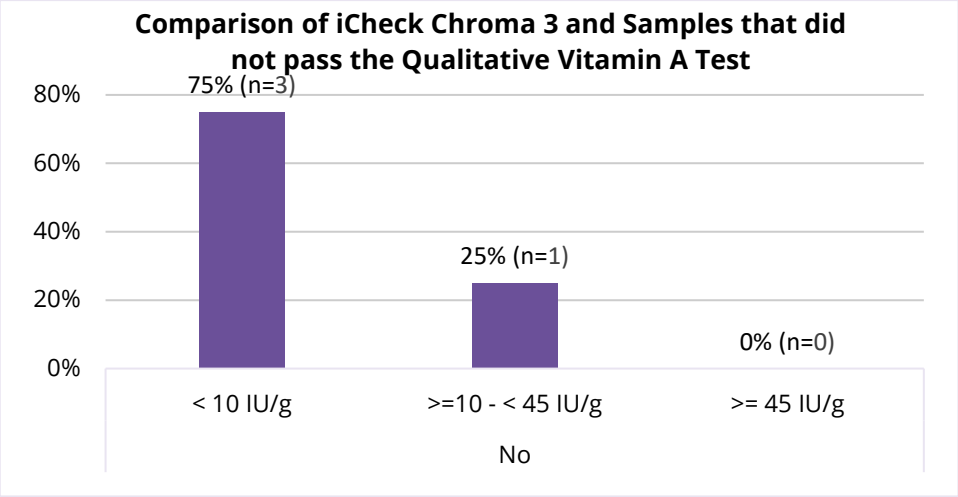


Figure 5: Comparison of iCheck Chroma 3 and qualitative vitamin A test results in edible oil samples. Samples did not pass the qualitative vitamin A test (no, n=4).

This distribution shows that the iCheck Chroma 3 device and spot tests generally agree on identifying the presence of vitamin A. Moreover, measurements with iCheck Chroma 3 enable the reader to accurately segment samples with vitamin A presence into two groups: adequately fortified samples and fortified below the national standard, and therefore,

provides to the analyst the information to make precise decisions in detecting fortification levels.

Following, 20% of samples measured with iCheck Chroma 3 were shipped to accredited laboratories for Vitamin A quantification. High Performance Liquid Chromatography (HPLC) was the method used in both reference laboratories, a local accredited laboratory and an external laboratory in Germany.

iCheck Chroma 3 recovery and precision, in combination with the data generated from an external laboratory was assessed using spiked control samples. This comparison was, however, not done with the local accredited by government laboratory.

Table 2: Vitamin A recovery (%) in spiked oils samples using iCheck Chroma 3 and the reference method External laboratory-Germany HPLC

Target concentration (added vitamin A at BioAnalyt lab)	iCheck Chroma 3	HPLC at External laboratory, Germany	HPLC at Local accredited laboratory
25 IU/g	26.33±1.33 IU/g (105% recovery) by BioAnalyt	21.6±0.1 IU/g (87% recovery)	Not sent
33.3 IU/g	36.33±0.13 IU/g (109% recovery) by BioAnalyt	Not sent	Not sent
43.3 IU/g	50.67±0.17 IU/g (117% recovery) by KFI analysts	Not sent	Not sent
43.3 IU/g	47.33±2.33 IU/g (109% recovery) by BioAnalyt	Not sent	Not sent
50 IU/g	48.33±2.33 IU/g (96% recovery) by BioAnalyt	43.3 IU/g (87% recovery)	Not sent

Method Comparison – iCheck vs. Local accredited laboratory-Indonesia

20% of collected samples were with HPLC at local accredited laboratory (n=100). Results with iCheck Chroma 3 indicated 84% of samples were fortified within national standard, 14% were below the recommended level, and 2% were below the LOQ. Similarly, the local accredited laboratory HPLC method showed 84% as adequately fortified, 8% below the national standard level, and 8% below the detection limit. These results demonstrate strong consistency between the two methods.

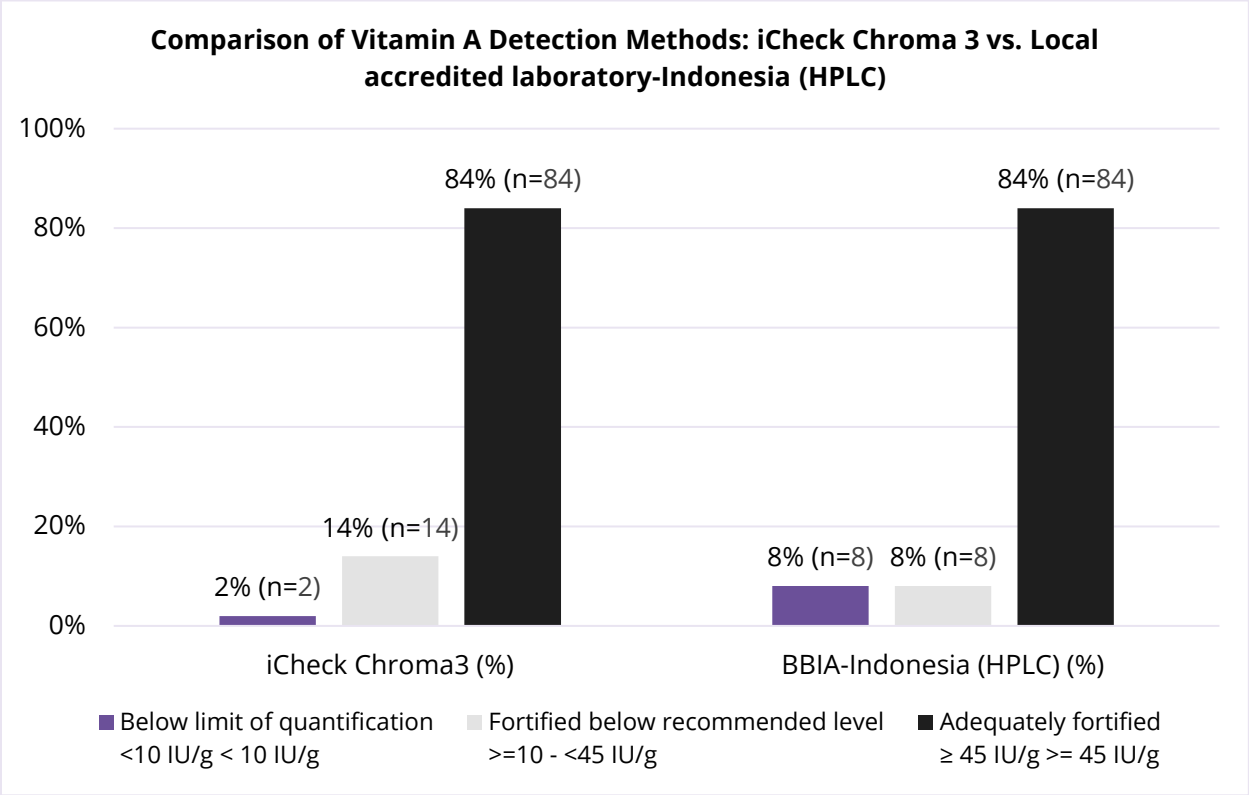


Figure 6: Comparison of Vitamin A Detection Methods: iCheck Chroma 3 vs. Local accredited laboratory (HPLC) (n=100, 20% of the total sampling).

To further expand this comparison, the following Bland-Altman plot compares the Vitamin A measurements between the local accredited laboratory HPLC method and iCheck Chroma 3. Briefly, the x-axis shows the average Vitamin A concentrations measured by both methods, while the y-axis displays the differences between these measurements. The solid black line represents the mean difference (bias), which trends slightly below zero, indicating that the iCheck Chroma 3 tends to slightly underestimate Vitamin A levels compared to the HPLC method. The dashed red lines signify the limits of agreement, calculated as ±1.96 standard deviations from the mean difference, providing a range within which most differences between the two methods are expected to lie:

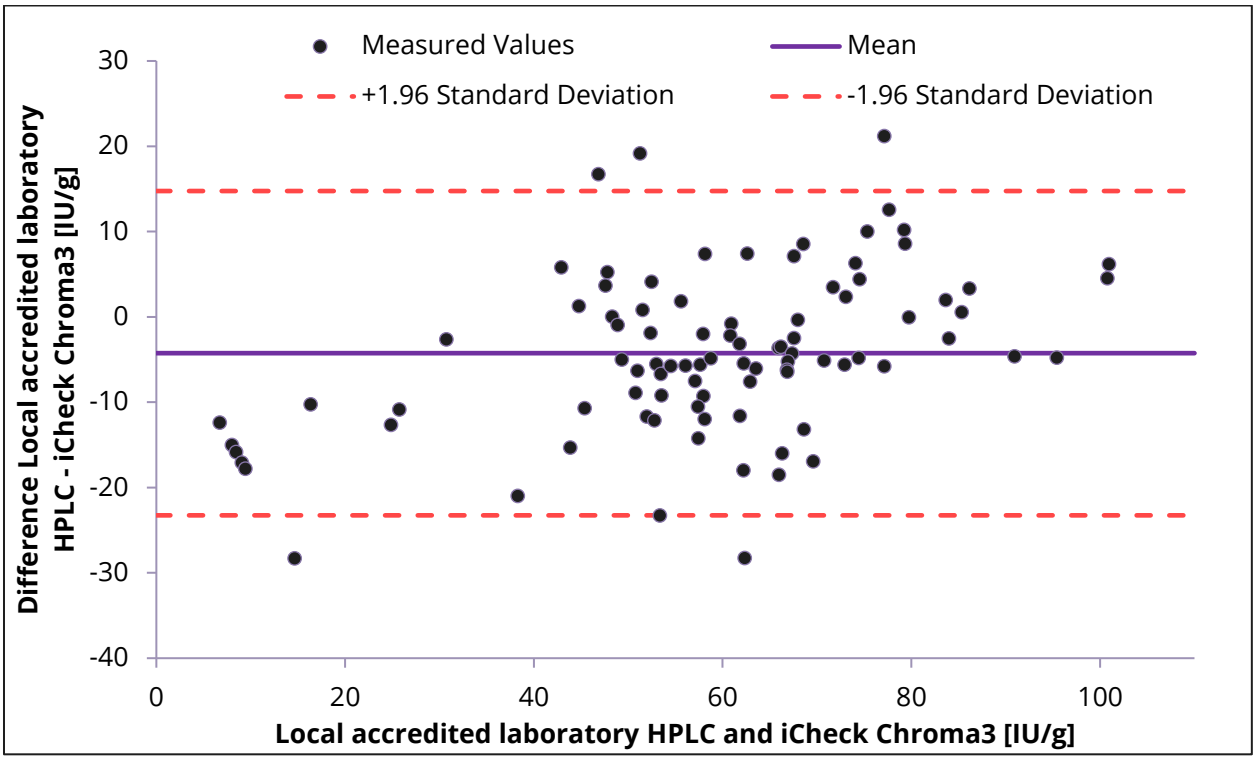


Figure 7: Comparison of the iCheck Chroma3 vs. Local accredited laboratory (HPLC) reference method.

The mean difference between the two methods is -4.3 IU/g, which suggests a slight overestimation by the iCheck Chroma 3 compared to the local accredited by government laboratory HPLC method. The standard deviation of 9.5 IU/g and limits of agreement ranging from -23 to 15 IU/g indicate that the variability between the two methods is within an acceptable range for most samples.

It is important to clarify that the samples analyzed are not identical, instead they are derived from the same batches, meaning that while individual samples are different, they originate from the same sampling set.

To support the above, the following linear correlation highlights that degree of agreement, with a Pearson correlation coefficient of 0.91, indicating that the iCheck Chroma 3 device provides results that closely align with the HPLC method. A high R^2value of 0.8283 indicates a strong correlation.

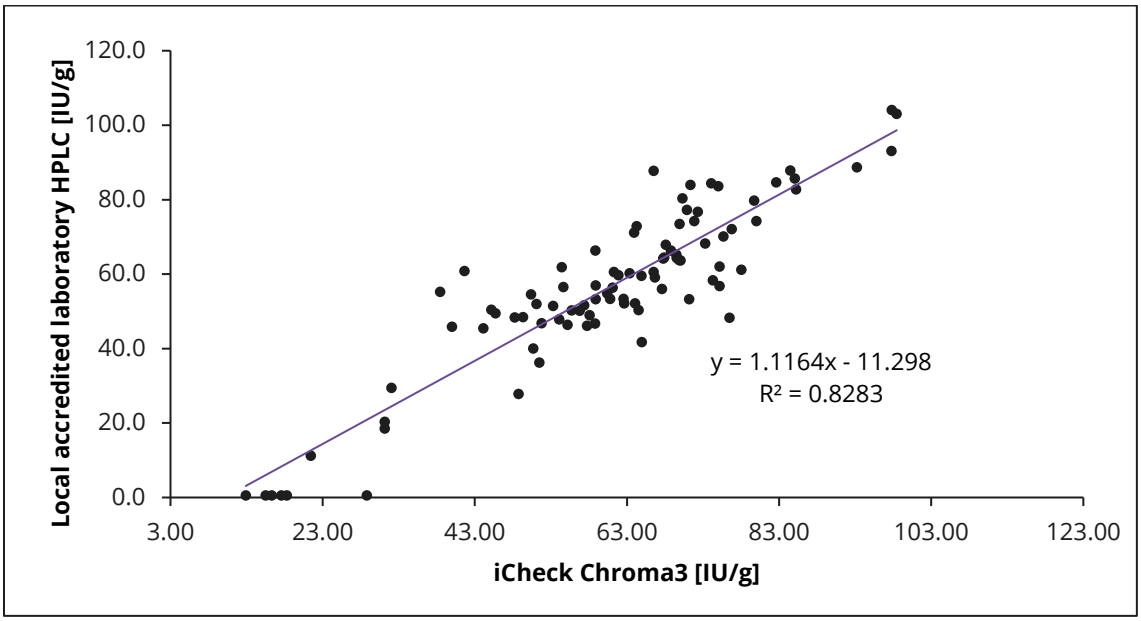


Figure 8: Linear correlation between the Vitamin A measurements from results obtained with iCheck Chroma 3 device and HPLC method performed by a local accredited laboratory.

Method Comparison – iCheck vs. External laboratory-Germany

In the following figure, a comparison of results obtained with iCheck Chroma 3 and with HPLC at an external laboratory (Germany) is displayed. The results indicate a high degree of consistency between the two methods in identifying adequately fortified samples. According to the iCheck Chroma 3 measurements, 86% (n=84) of the samples were adequately fortified, 14% (n=14) were fortified below the recommended level, and 0% (n=0) were below the limit of quantification. Similarly, the External laboratory-Germany HPLC method reported that 60% (n=84) of the samples were adequately fortified, 30% (n=29) were fortified below the recommended level, and 10% (n=10) were below 10 IU/g. These results demonstrate an alignment between the method iCheck Chroma 3 and the conventional HPLC method from the external laboratory.

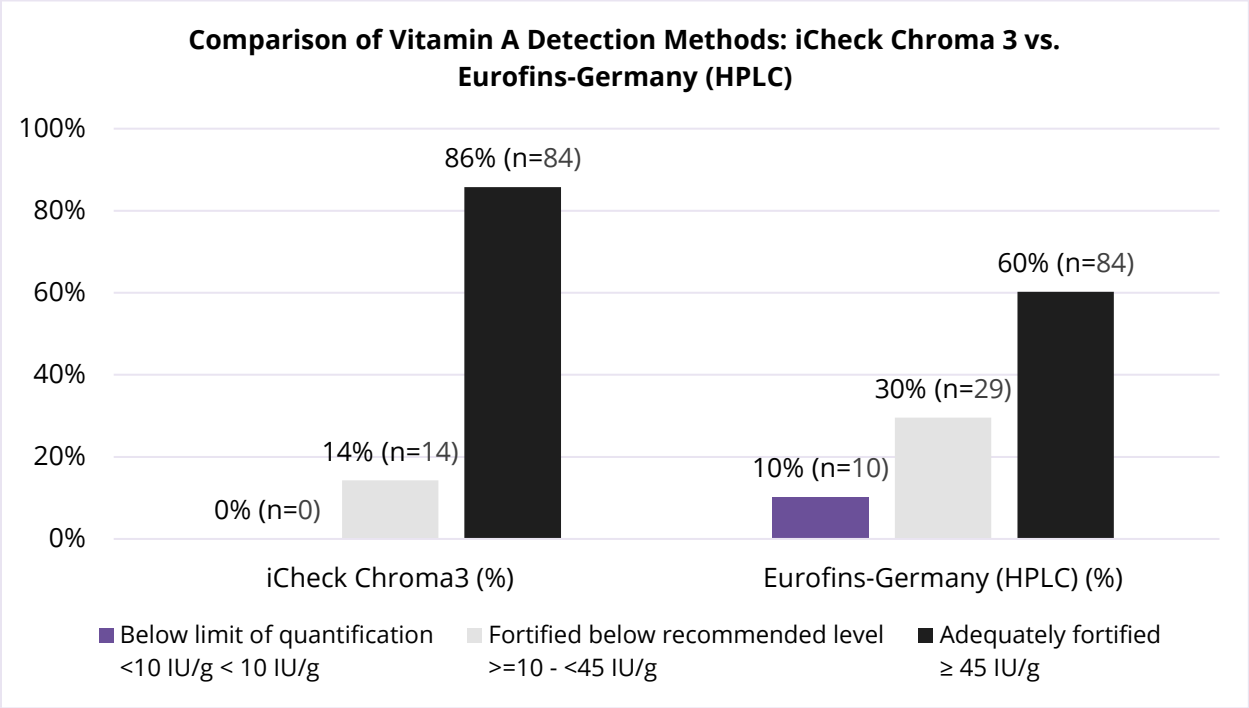


Figure 9: Comparison of Vitamin A Detection Methods: iCheck Chroma 3 vs. HPLC External laboratory-Germany (n=98, approximately 20% of the total sampling).

The Bland-Altman plot comparing the External laboratory-Germany HPLC method with the iCheck Chroma 3 device reveals good overall agreement, like the previous comparison with a local accredited laboratory.

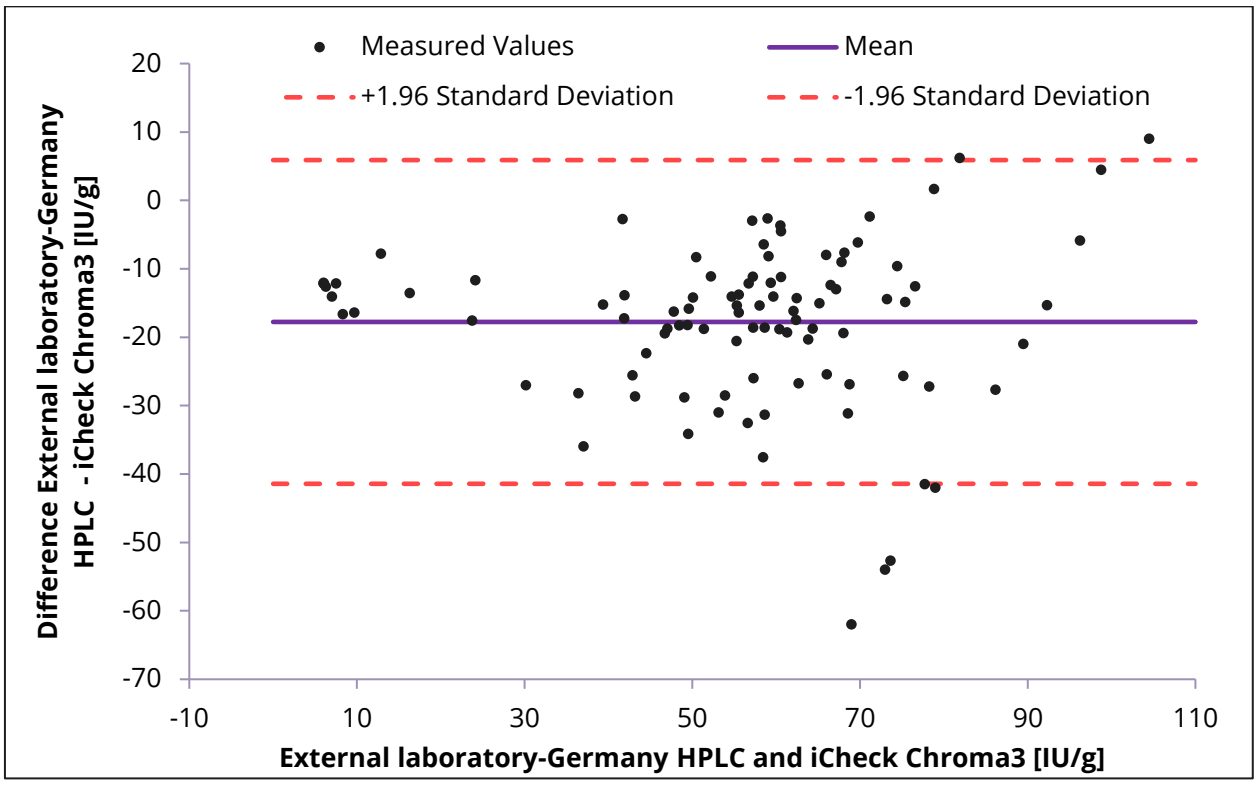


Figure 10: Comparison of the iCheck Chroma3 vs. External laboratory-Germany (HPLC) reference method.

The correlation plot between the External laboratory-Germany HPLC method and the iCheck Chroma 3 demonstrates a positive linear relationship between the two sets of measurements, with an R2 value of 0.73 and Pearson of 0.86 indicating a moderate to strong correlation.

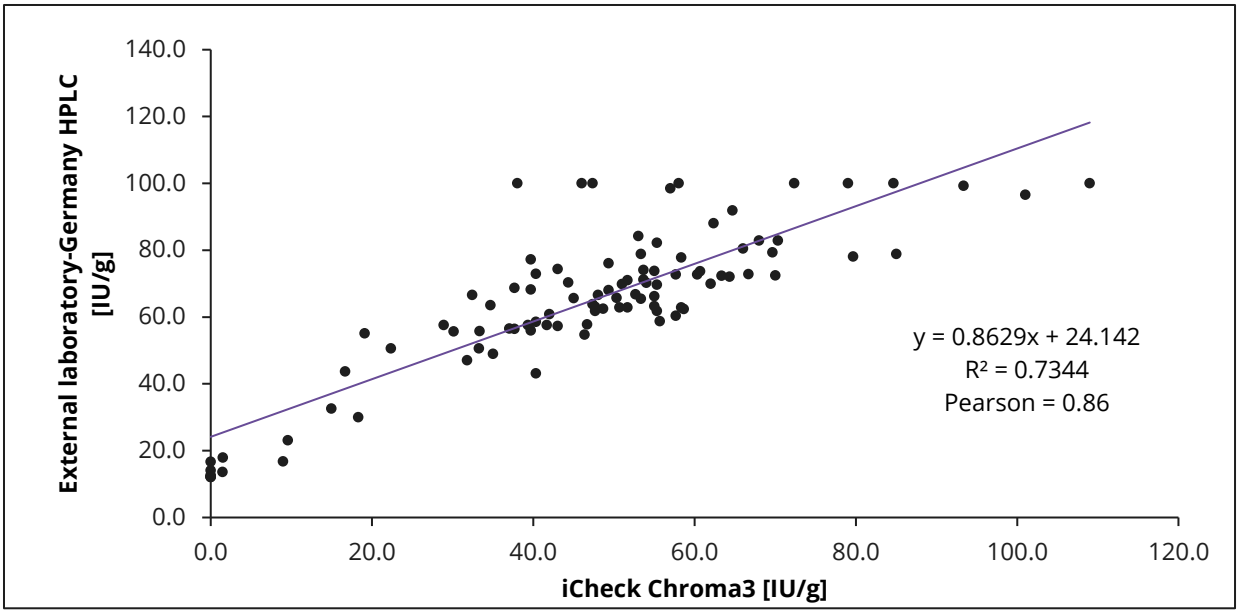


Figure 11: Linear correlation between the Vitamin A measurements from the iCheck Chroma 3 device and the External laboratory-Germany HPLC method.

Overall, the comparison between traditional qualitative methods like HPLC and iCheck results showed a strong agreement. The iCheck Chroma 3 device demonstrated a strong agreement with the local accredited laboratory HPLC method, and moderate agreement with the external laboratory-Germany HPLC method. The moderate agreement with External laboratory HPLC could be explained by the lower recovery by External laboratory method of vitamin A in edible oil samples of 87-88%, whereas iCheck Chroma 3 method has a tendency for overestimation at 96-117%. However, all methods had comparable performance in terms of identifying the results according to national standard specifications.

Overall, the comparison between traditional qualitative methods like HPLC and iCheck Chroma 3 results showed a strong agreement. The iCheck Chroma 3 device demonstrated a strong agreement with the local accredited laboratory HPLC method. A similar comparison between iCheck Chroma 3 and results obtained from an external laboratory by HPLC are added in the Annexes (see Annexes).

The iCheck Chroma 3 method demonstrated high consistency with the traditional HPLC methods, with most samples classified similarly across non-fortified, fortified below the national standard, and adequately fortified. This consistency underscores the reliability of iCheck Chroma 3 as a rapid quantitative test for vitamin A fortification.

From a cost perspective, iCheck Chroma 3 offers substantial savings, with an analysis cost of less than 10 EUR per sample, compared to 25 EUR per sample for the local accredited

laboratory and 127 EUR for testing at an external laboratory-Germany. This economic advantage makes iCheck Chroma 3 a viable option for large-scale monitoring programs.

For in depth analysis of the results in terms of the levels by brand, sales location and geography please refer to the report prepared by KFI and NI.

85% of all collected samples were branded and belonged to 9 brands that capture 85% of the packaged oil market that falls under the mandatory fortification regulations. The remaining 15% were “Minyakita "subsidized" packaged oil. Each brand was collected between 22 to 97 times – this was done to investigate the differences between different types of shops and the two cities Jakarta and Surabaya. The sample number could be significantly decreased by either creating composite samples or just reducing the number of samples to, for example, 2-3 batches of the same brand per location. Those samples could be pooled into one for the actual testing.

INDONESIA: LIMITATIONS AND CHALLENGES DURING PLANNING AND IMPLEMENTATION

Challenges	Impact on	Control Measures & Mitigation	Recommendations
Limited Market Representation and Sampling Issues: 70% of cooking oil is unpackaged, with only 30% labeled and fortified.	Difficulties to ensure a scope that could cover 85% of available edible oil reaching the local population.	Our approach focused on select brands (recommended by KFI) where fortification is regulated. A subsidized product, 'Minyak Kita', was added to the sample selection, which accounts for 26% of the total market (packed + unpacked oil). Commonly provided by each producer as a cheaper alternative to branded and packaged oil.	Consider integrating local market information and account for market specific dynamics. Set realistic expectations when sizing market to facilitate sampling by local partners and account the limitation the market (only 30% of the total local oil market could be sampled).
Sample collection could only take place in January to ensure testing happens within 4 weeks of sample collection.	Calendar: testing phase	Quality of Vitamin A measurements. Proposed collection dates: end-Jan 2024. Proposed testing early-Feb 2024.	Establish hard deadlines at the kickoff meeting involving the input of the local collection team. Coordination with established collection calendars is essential for ensuring self-organization within the team.
Local team (KFI) is unable to collect samples with different LOTs for each brand on the same date/store. KFI cannot	Sample selection	Proposed collecting samples on different dates. Proposed: verify at least 3 different LOTs from samples to be collected.	Appoint a contact lead from the local stakeholder to manage communications and set realistic expectations

guarantee this type of collection.			regarding the diversity of samples that can be collected.
Issues with importation of iChecks and kits into the country. Several factors: Lack of experience to import/export via temporary licence with local distributor in Indonesia.	iChecks and Kits Shipment	The distributor can ship using CIP (to the airport and around), followed by invoicing for import costs and internal shipment. Importation via the normal process (full taxes), where the distributor purchases in advance at lower prices. Post-assessment, the devices remain with the distributor for internal sales.	Review if the distributor has an updated importation license. Consider different importation methods: 1. Transporting iChecks with analysts from Germany. 2. Direct sale to the distributor through normal importation. 3. Temporary import/export for demos and tradeshows.
Local partner sent 20% of samples for HPLC analysis at BBIA in advance to the onsite limiting our capacity to prepare a distributed sampling for comparison of methods with HPLC in Indonesia and Germany	Data generation	Coordinate better with local partners to ensure all samples are available for the planned testing schedule. Improve pre-arrival communication and planning to avoid premature sample dispatch.	Ensure clear communication protocols and schedules with local partners. Implement a centralized tracking system for sample collection and dispatch to avoid premature or misaligned sample handling.
KFI and NI generated a sampling protocol based on market knowledge, targeting 85% of commercial brands in Indonesia, but the number of samples could have been reduced to avoid multiple repetitions.	Sample collection scope	Proposed a sampling reduction plan and improved coordination to streamline the number of samples collected to avoid redundancy.	Enhance communication and coordination before signing contracts. Establish a more efficient sampling protocol that balances comprehensive coverage with logistical feasibility, aiming to reduce redundancy and ensure high-quality data collection.
Outsourcing of chemicals locally reported to be a time-consuming and difficult to coordinate process. Nonetheless, it enabled us to avoid generating documentation for importation in the country, which given the nature of the products, is complex and varies to local legislation.	Qualitative testing preparation	Mitigation included procuring extras and utilizing help from local partners for local sourcing of necessary chemicals.	Strengthen local partnerships to facilitate the sourcing of chemicals and streamline importation processes.

2. KENYA

BACKGROUND

Wheat and maize flour are staple foods in Kenya, consumed daily by a large portion of the population. Fortifying these flours ensures that a broad demographic, including vulnerable groups such as children and pregnant women, receives adequate micronutrients, thus improving immune function, reducing the incidence of infectious diseases, and enhancing overall health and development. Large-scale fortification programs have demonstrated substantial improvements in vitamin A intake and status, leading to reductions in deficiency-related health issues.

Over the last decade, Kenya has been implementing mandatory fortification of staples (maize and wheat flour), with essential vitamins and minerals, to address micronutrient deficiencies. These regulations are detailed in Kenya Standards KS EAS 768 for maize flour and KS EAS 767 for wheat flour, specifying the inclusion of vitamin A, iron, zinc, and B vitamins to improve public health outcomes. Fortifying these staple foods is a sustainable and cost-effective strategy to combat malnutrition and enhance the nutritional quality of widely consumed foods.

Kenya's flour market is characterized by a mix of large, medium, and small-scale millers, where a small group of key players dominate the market, holding substantial milling capacities. TechnoServe unpublished reports mention a high market consolidation for wheat flour, with five leading mills holding over 60% of the market share. For maize flour, the top 23 mills hold 55% of the market share.

Moreover, results of recent national surveys at industry levels surveillance on food fortification in wheat and maize flour between 2023 and 2021, describe a decrease in the

level of fortification from 46% maize and 84% for wheat flour in 2021, to 42% and 75%, respectively by 2023 (MOH/NI/JKUAT 2021 and 2023). This indicates significant room for improvement in compliance and fortification practices among producers.

Furthermore, Technoserve unpublished data denotes that 11% of wheat flour target market complied with fortification standards, while for maize flour, 19% of the market is known to meet the requirement standards for fortification.

The specific requirements for fortifying wheat and maize flour in Kenya are as follows:

- Fortified Wheat Flour (KS EAS 767):
    - Vitamin A (Retinyl palmitate, spray-dried or equivalent): Minimum 0.5 mg/kg and maximum 1.4 mg/kg, tested using AOAC 2001.13.
    - Total Iron: Minimum 20 mg/kg tested using AOAC 944.02

- Fortified Maize Flour (KS EAS 768):
    - Vitamin A (Retinyl palmitate, spray-dried or equivalent): Minimum 0.5 mg/kg and maximum 1.4 mg/kg, tested using AOAC 2001.13.
    - Total Iron: Minimum 21 mg/kg tested using AOAC 944.02

By implementing these regulatory requirements, the government of Kenya aims to guide fortification practices to enhance the nutritional quality of flour products. This initiative is a significant step towards mitigating micronutrient deficiencies and promoting better health outcomes in the population.

SAMPLE COLLECTION

TechnoServe, the local implementing partner in Kenya, led the efforts in contacting producers and collecting samples of wheat and maize flour across the country. It is important to note that the entire set of samples was part of an internal study by TechnoServe. Consequently, the samples received, labeled with internal codes, are included in that study.

To protect miller interests and maintain business relationships, all samples and data were anonymized, including the omission of customer names. For this project, TechnoServe collected 126 packages of individual brands. Each package was a composite of three packets, yielding a total sample weight of 120 grams, where 87 were marked WHF (wheat flour), and 39 as MZF (maize flour).

In terms of market representativity, TechnoServe ensured that samples were collected from brands processed by the members of the Cereal Millers Association (CMA), with whom they collaborated. CMA allied millers represent brands covering over 80% of the wheat flour sector and approximately 40% of the maize flour sector in Kenya. The maize sector is particularly fragmented, with micro and small-medium-sized enterprises holding a significant market share despite their seasonality. Due to the early nature of the sample collection, maintaining the quality of the samples over several months until the testing phase

posed a challenge. To address this, refrigeration equipment was procured locally to store the samples at a controlled temperature of approximately -20°C.

TRAINING METHODOLOGY

The study in Kenya aimed at assessing levels of vitamin A and iron in wheat and maize flour samples. Two trainers from BioAnalyt trained two lab analysts at the hosting facility, the African Millers School, in Nairobi. The training methods included both qualitative colorimetry testing for Vitamin A and Iron in Flour, and quantitative testing using our iCheck technology.

At the beginning of the session, the trainers provided a comprehensive guide on sample handling procedures and described the protocols for qualitative colorimetric testing for both Vitamin A and Iron in wheat and maize flour. This included the preparation of the necessary solutions.

Following this, an in-depth introduction to the iCheck Fluoro and iCheck Iron devices was given, covering step-by-step calibration of the devices, sample preparation, readout, and interpretation. Results of the training session in Indonesia are displayed in Annexes. Furthermore, detailed instructions for each technique are outlined in the following sections.

TESTING METHODOLOGY

In Kenya, the qualitative testing methodology for analyzing vitamin A and iron in wheat and maize samples required the local procurement of chemicals for spot testing implementation. Procuring these chemicals online or through our local distributor posed significant challenges, even for small volumes of some reagents. In this context, we received support from AMS for the local procurement in Nairobi.

The qualitative colorimetric test allowed analysts to provide a preliminary assessment of presence of vitamin A and iron in wheat and maize flour. For vitamin A, the readout of this experiment resulted in a Yes/No decision based on the visual evaluation of a blue color formation, indicating the presence of vitamin A. Additionally, beyond a Yes/No evaluation for the presence of iron, this chemistry-based assay enabled the identification of specific types of iron, such as ferrous fumarate or NaFeEDTA.

Subsequently, a quantitative analysis was conducted using the iCheck Fluoro device to obtain precise measurements of vitamin A content, and the iCheck Iron device was used to measure total iron from the same sample. To ensure quality control, spiked control samples of wheat flour were analyzed after every tenth sample, and every tenth sample was analyzed in duplicates using the same dilution protocol.

At the end of this workflow, approximately 20% of the analyzed samples were sub aliquoted for further verification using reference methodologies in accredited laboratories. Approximately 32 samples were collected in duplicates sent to a local accredited laboratory in Nairobi, and an external laboratory Germany. It is important to note that the samples chosen for analysis in Kenya and Germany were the same, as they were collected in duplicates from the original package received from TechnoServe.

This rigorous testing framework ensures a comprehensive evaluation and validation of the fortification levels in wheat and maize flour samples. The following section will briefly describe the methods used during this study.

QUALITATIVE TESTING: Colorimetric Assay

The protocol for qualitative testing of vitamin A is based on the lab methods published by ECSA-HC in 2017 and iron qualitative test is based on AACC method 40-40 as described by Reddy et al, 2020. The following section covers both qualitative colorimetric analyses used to screen for the presence of Vitamin A and Iron in flour and maize samples:

Colorimetric assay - VITAMIN A in Flour

The colorimetric qualitative analysis for vitamin A in flour samples involves adding a chromogenic reagent to react with retinol, producing a blue color complex proportional to the retinol concentration. For flour samples, the process includes extracting vitamin A from the solid matrix. The protocol was optimized to reduce the volume of reagents as compared to SOP from ECSA manual by 90%.

Solution Preparation

- Solution 1: Prepare supersaturated TFA (Trifluoroacetic Acid) in DCM (Dichloromethane) solution. The TFA in DCM solution dissolves the extracted oil from the flour sample and aids in the reaction with the chromogenic reagent, producing a more distinct color change.
- Solution 2: Prepare working solution volume with 2-propanol and n-heptane. This combination ensures efficient extraction of retinol from the aqueous phase into the organic phase, facilitating an accurate colorimetric reaction.

To prepare the above solutions, the following chart provides a reference based on the number of samples to be analyzed, along with the corresponding calculated dilutions for the chemical reagents:

Table 3: Dilution chart for solution preparation to prepare a TFA/DCM based qualitative colorimetric testing.

	Chemical	Number of Sample						Units
		1	10	20	50	100	200	
Solution 1	TFA	0,2	2	4	10	20	30	mL
	DCM	2	20	40	100	200	300	mL
Solution 2	2-propanol	1	10	20	50	100	150	mL
	n-heptane	1	10	20	50	100	150	mL

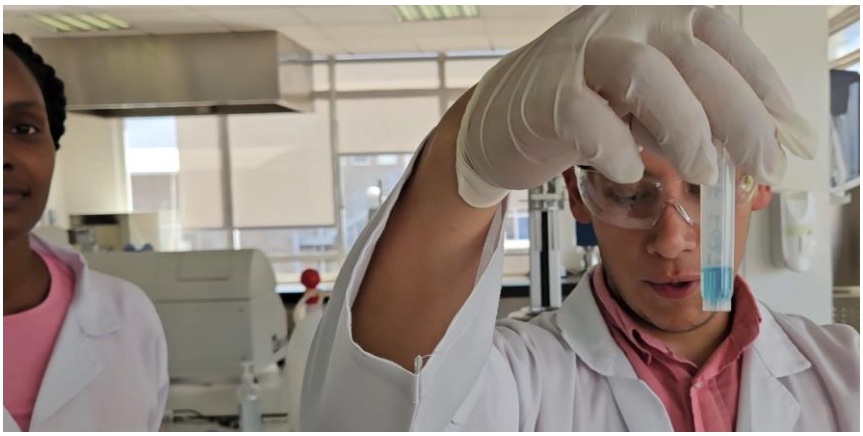
The use and handling of these chemicals require protective gloves and goggles to prevent severe skin irritation. These solutions remain stable for two weeks when refrigerated.

Sample Analysis

- Step 1**:** Weigh out 2 g of wheat flour to a 15ml Falcon Tube. (Note: When using maize flour use 4g).
- Step 2**:** Add 2 mL of Solution 2 to the sample and vigorously mix by hand, ensuring complete integration by taping the container against the table if necessary. Add 2 mL of water and shake vigorously again.
- Step 3: Add a pinch of salt and shake again. Next centrifuge for 10 seconds (use the provided manual centrifuge with caps). Notice the formation of a supernatant liquid layer.
- Step 4: Add 0.6 mL from Solution 1 to the Eppendorf Tube, and then, transfer 0.2 mL of the supernatant from STEP 3 and mix. Observe color change within 5 seconds.

If blue color appears then it is positive for vitamin A, if no blue color it is negative for vitamin A presence in flour.

The use of TFA in DCM for vitamin A analysis in flour offers a strong reaction, while solvent of 2-propanol and n-heptane provides more efficient phase separation and better stabilization of retinol in the organic phase. However, important limitations in this method are the transient nature of the reaction, difficulties to obtain reliable interpretation of the blue color, especially with low levels of Vitamin A as per standard which can provoke variability during analysis and increase the appearance of false positive/negative results.



Colorimetric assay – IRON in Flour

Solution Preparation

- Solution 1: Prepare a 2-N Hydrochloric acid (HCl) solution using distilled or bottled water. This solution will aid in breaking down the sample matrix and releasing iron ions into solution.
- Solution 2: Prepare a 10% Potassium Thiocyanate (KSCN) solution in distilled or bottled water. Potassium thiocyanate reacts with iron ions to form a colored complex, making the presence of iron visible.
- Solution 3: Mix equal parts of Solution 1 and Solution 2. This mixture combines the acid medium necessary for the reaction and the reagent that forms the colored complex with iron ions.
- Solution 4: Hydrogen Peroxide solution is usually supplied in the necessary concentration and requires no further preparation. It is often used to oxidize any iron present to its detectable form.

Sample Analysis

- Step 1: Spread flour on a flat surface into a thin layer. One can smooth out the area with a beaker.
- Step 2: Apply Solution 3 by dropping. Wait 2 minutes for the appearance of red dots, indicating the presence of ferric iron (i.e. NaFeEDTA). If no red dots appear, the sample might still contain ferrous iron (i.e. ferrous fumarate) that could be detected with the

aid of hydrogen peroxide. The following image illustrates two samples without content of IRON (left panel) and visible red dots detecting IRON content (right panel).

- Step 3 (Optional): Apply Solution 4 for ferrous iron detection. Look for red dots within 2 minutes.

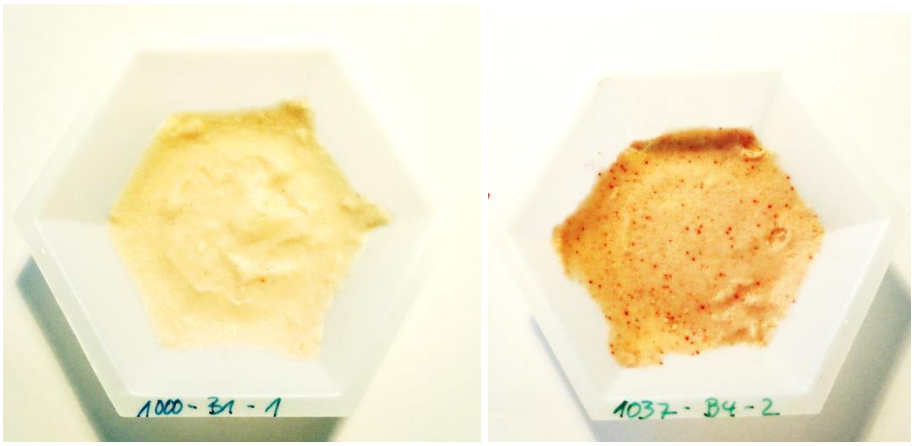
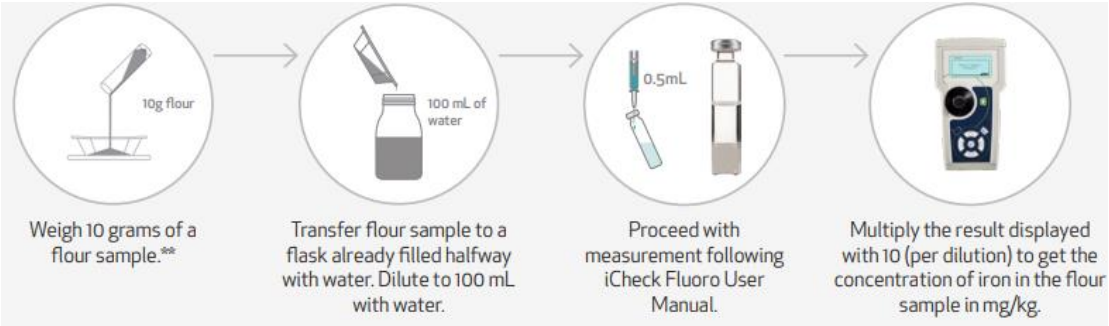


Figure 12: Qualitative Analysis of the Presence of Iron in Wheat Flour Samples. Control samples without iron content are shown in the left panel, where no red dots were found. In contrast, the right panel shows the formation of visible red dots, indicating the presence of iron (NaFeEDTA). When no dots are detected, it is optional to spread a hydrogen peroxide solution to detect ferrous iron.

Quantitative analysis of VITAMIN A and IRON in Flour with iCheck devices

For the determination of **vitamin A in flour, iCheck Fluoro** was used, a portable, single wavelength fluorometer that quantitatively measures vitamin A in foods and biological substances by measuring added vitamin A as retinyl palmitate and retinyl acetate. The principle involves the excitation of retinol at a specific wavelength of 325 nm, resulting in the emission of light at a different wavelength. The iCheck Fluoro measures this fluorescence intensity, calculating the vitamin A content through an algorithm and displaying the result in µg retinol equivalents (RE)/L. Results are stored in the device and can be transferred to a computer.

When measuring vitamin A in flour, it is recommended to first measure an unfortified sample of the same flour to assess if the food matrix has innate fluorescence. This may cause iCheck Fluoro to display overestimated results. The following is the sample preparation and analysis workflow for Vitamin A measurement:



A brief review description of how to dilute the sample for measurement is shown in the following iCheck Flour training video: https://www.youtube.com/watch?v=D6KioAzQxw4.

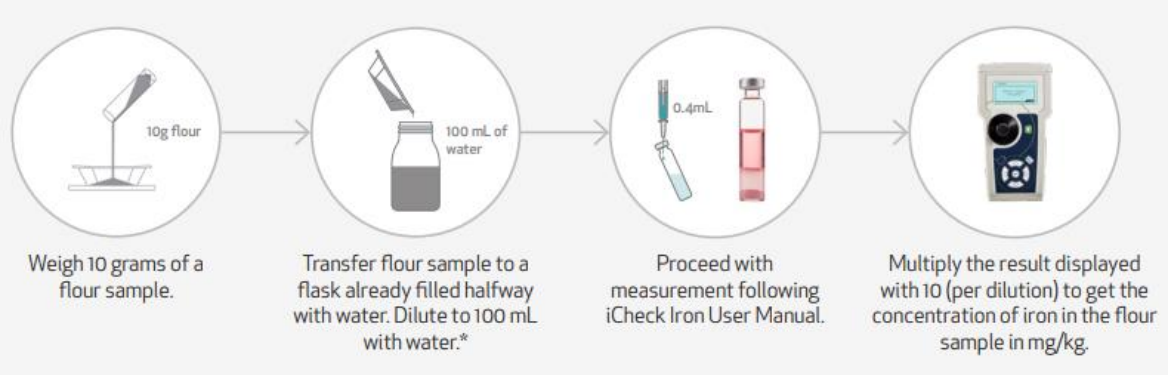
Additional information on how the measure is taken can be found in the following training video: https://www.youtube.com/watch?v=2Vh2yAdv-Fc

The wheat and maize flour samples were diluted 1:5 with bottled/distilled water to ensure the final solution's vitamin A concentration was within the iCheck Fluoro linear range of 50-3000 µg RE/L. The diluted samples were injected, incubated for 5 minutes, centrifuged, and measured with the iCheck Fluoro. Unfortified samples of local wheat and maize flour were measured with iCheck Fluoro to assess innate fluorescence. The results obtained with iCheck Fluoro with market samples were corrected for this innate fluorescence (0.6 mg/kg for both wheat and maize flour).

The **iCheck Iron** is a portable, single-wavelength photometer, that quantitatively measures iron in multiple food matrices based on colorimetric detection. The iCheck Iron measures absorption at 525 nm, using reagents containing bathophenanthroline in organic solvent, along with reducing and chelating agents. The red color intensity correlates with the iron concentration. This intensity is measured by the device and converted to iron content, displayed in mg iron/kg of sample.

For sample preparation, flour samples are injected into a reagent vial prefilled with a chromogenic reagent, mixed, and then measured with the iCheck Iron device. The device measures color intensity at specific wavelengths for accurate iron quantification. Results are stored in the device and can be transferred to a computer.

For sample preparation, injection into a reagent vial and measurement in the device the following workflow was followed:



Importantly, for ferrous fumarate, ferrous sulphate, and ferric pyrophosphate, it is recommended to dilute flour sample in 0.2M HCl solution since these iron compounds are only partially soluble in water. NaFeEDTA is soluble in water, hence water can be used as a diluent. It is recommended also to measure intrinsic iron in flour samples using 0.2M HCl. Intrinsic iron is natural iron present in organic samples. In flour the intrinsic iron content may be between 5 and 60 mg/kg, the higher the bran content the higher the level of intrinsic iron.

The wheat and maize flour samples were diluted 1:10 with 0.2M hydrochloric acid to ensure the final solution's iron concentration was within the iCheck Iron's linear range of 1.5-12.0 mg Fe/L. The diluted samples were injected, incubated for one hour, centrifuged, and measured with the iCheck Iron.

A brief review description of how to measure the sample is shown in the following iCheck Iron training video: https://www.youtube.com/watch?v=cW6jUOXOAmQ&t=501s.

QUANTITATIVE TESTING: Reference laboratories

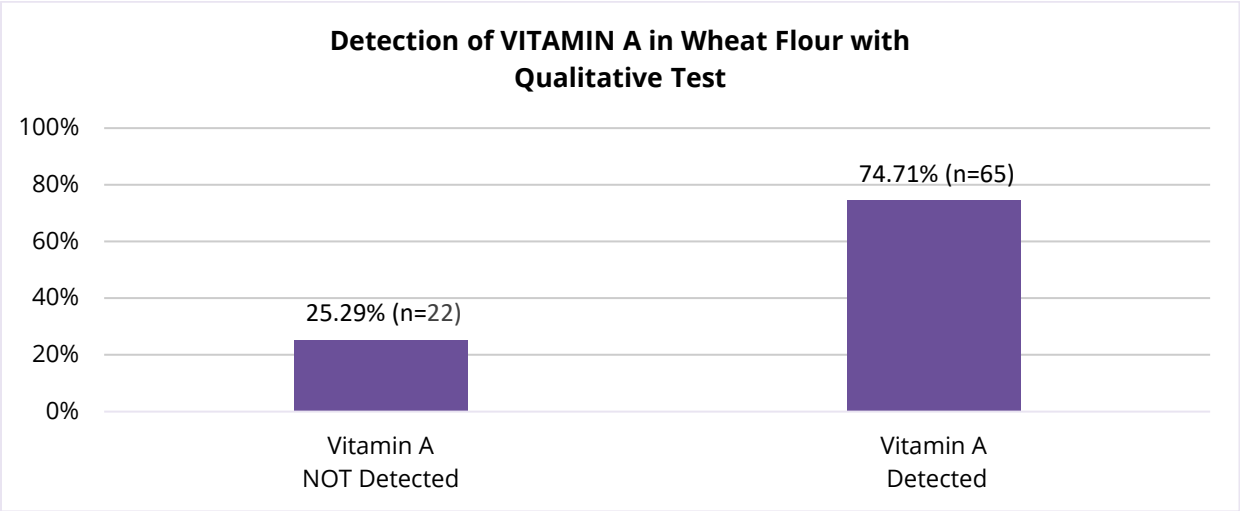
To ensure the reliability and accuracy, approximately 20% of samples measured with iCheck Iron and Fluoro were shipped to accredited laboratories for Vitamin A and Iron quantification. High Performance Liquid Chromatography (HPLC) was the method used in both reference laboratories, an external laboratory in Germany (DIN EN 12823-1. - HPLC/Fl) and a local accredited laboratory in Kenya. Similarly, for measuring total iron levels in flour samples, the method Inductively coupled plasma mass spectrometry (ICP/MS) was used at the external laboratory in Germany (DIN EN 15763, mod. - ICP/MS) and atomic absorption spectrometry (AAS) at a local accredited laboratory.

RESULTS

Determination of Vitamin A in wheat and maize samples

A total of 144 samples of wheat and maize flour were analyzed through a qualitative vitamin A test. Most **wheat flour** samples (75%, n=66) tested positive for vitamin A, with only 25%

(n=22) showing no detectable levels. In contrast, the majority of **maize flour** samples (82%, n=32) did not detect vitamin A, while only 18% (n=7) tested positive.



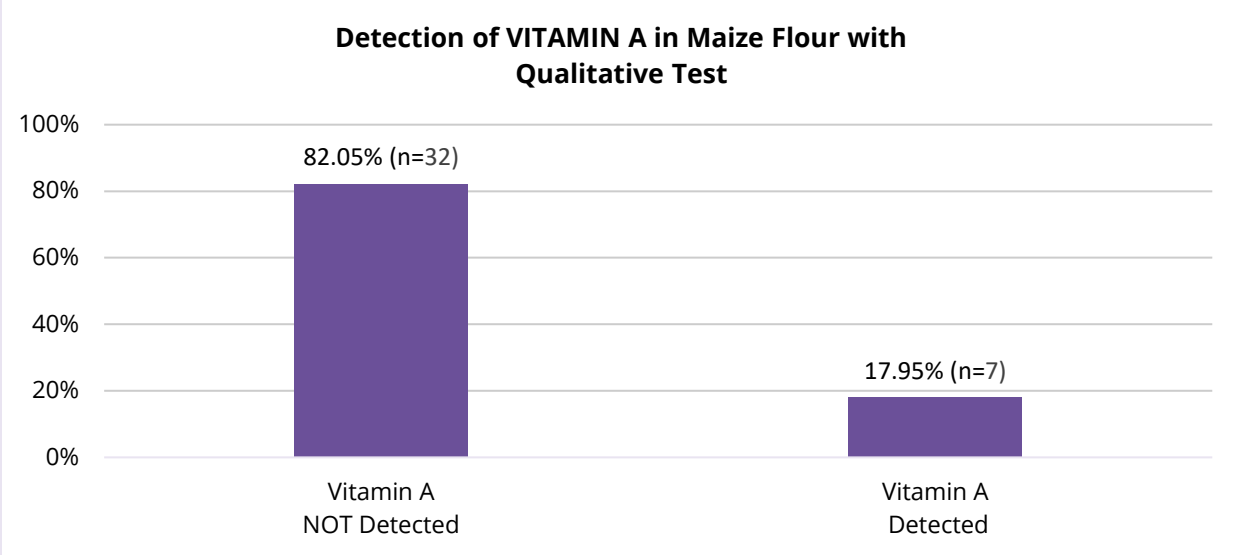


Figure 13: Qualitative vitamin A test in wheat (above, n=87) and maize flour (below, n=39).

Kenya has a national standard for fortification with Vitamin A in wheat flour and in maize flour of 0.5 – 1.4 mg/kg. Considering that iCheck Fluoro linear range is 50-3000 µg RE/L (0.05-3 mg/kg) and dilution factor of 5, results with iCheck were grouped in the following way:

- Below LOQ: <= 0.25 mg/kg
- Fortified below recommended level: 0.05 - < 0.5 mg/kg
- Adequately fortified: >= 0.5 - <= 1.4 mg/kg
- Fortified above recommended level: > 1.4 mg/kg

The quantitative analysis using iCheck Fluoro shows that in wheat flour samples, 22% (n=19) are fortified below the recommended level, 22% (n=19) are adequately fortified, and 5% (n=4) are fortified above the recommended level. Additionally, 52% (n=45) of the samples are

below the LOQ. In maize flour samples, 33% (n=13) are fortified below the recommended level, 5% (n=2) are adequately fortified, and one was fortified above the recommended level, with 59% (n=23) being below the LOQ. These findings suggest that a significant proportion of both wheat and maize flour samples do not meet the recommended fortification levels with vitamin A, with a notable number of samples falling below the quantification limit.

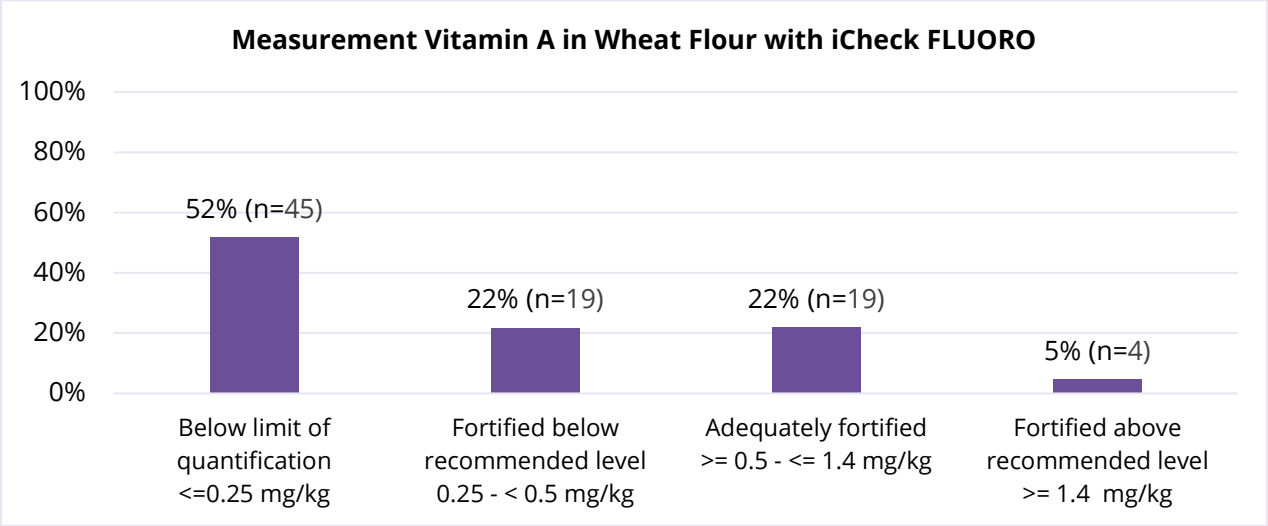


Figure 14: Measurement Vitamin A in wheat (n=87) flour with iCheck Fluoro.

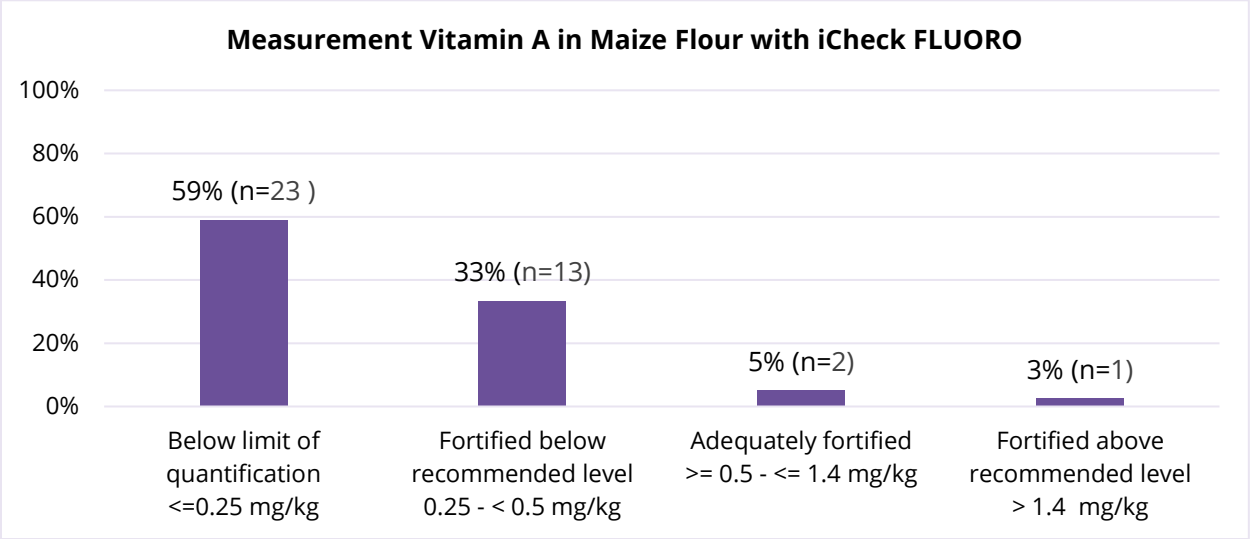


Figure 15: Measurement Vitamin A in maize (n=39) flour with iCheck Fluoro.

11 wheat flour samples that measured >0.25 mg/kg with iCheck Fluoro were assessed as not fortified with qualitative assay. Conversely, 35 samples that measured <0.25 mg/kg with iCheck Fluoro were assessed as fortified.

10 maize flour samples that measured >0.25 mg/kg with iCheck Fluoro were assessed as not fortified with qualitative assay. Conversely, 2 samples that measured <0.25 mg/kg with iCheck Fluoro were assessed as fortified. These findings underscore the variation and potential inaccuracies in the qualitative spot test with such low vitamin A levels.

Method Comparison – iCheck vs. Local accredited laboratory-Kenya

32 wheat and maize samples were analyzed also at the local government-accredited laboratory for vitamin A testing in flour using HPLC. The results were compared to those obtained with iCheck Fluoro. The proportion of adequately fortified samples is similar for the 2 methods, although iCheck Fluoro tends to overestimate, likely due to matrix effect.

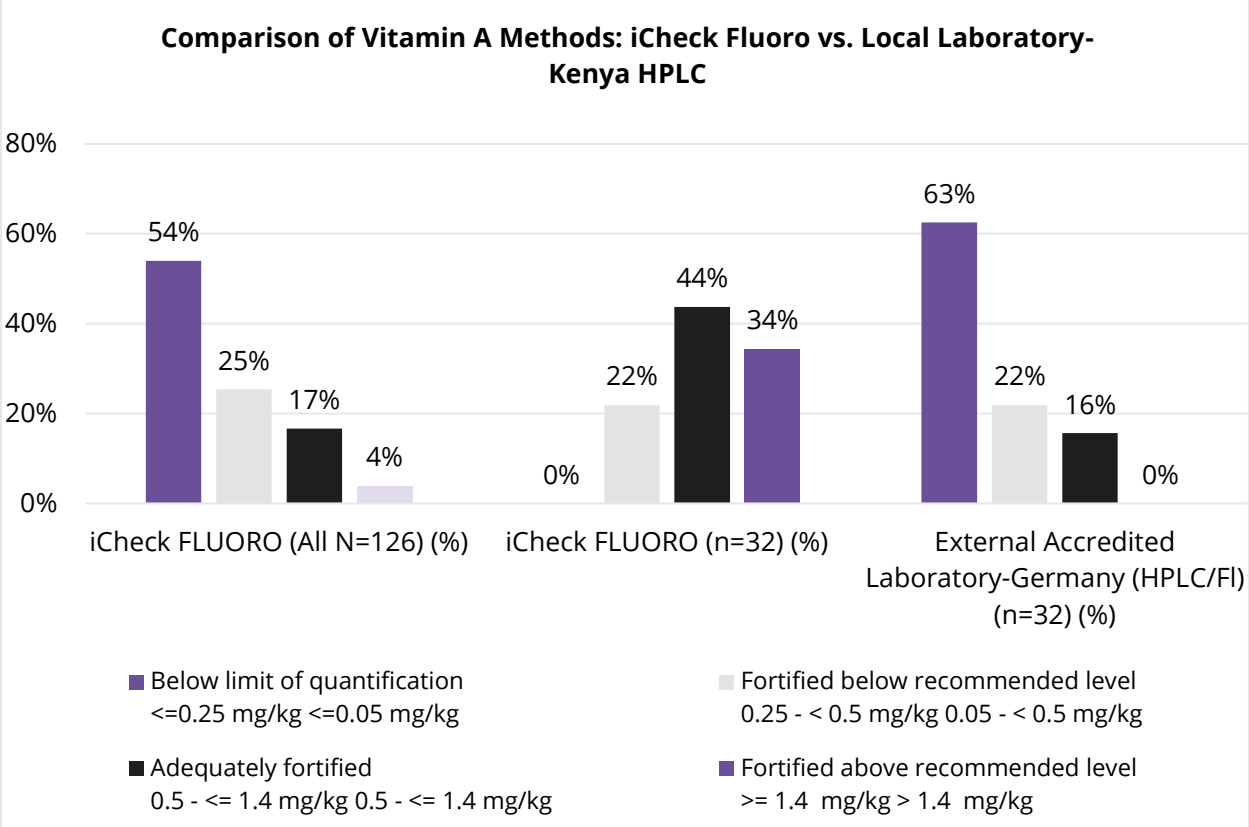


Figure 16: Comparison of Vitamin A levels as assessed with different methods: iCheck Fluoro for all samples (n=126) and same samples as analyzed with HPLC (n=32) vs. local accredited laboratory-Kenya HPLC (n=32).

Vitamin A results obtained at the Local accredited lab with HPLC method and iCheck Fluoro were further compared. Bland-Altman analysis shows a mean difference of -0.35 mg/kg,

indicating a small bias where iCheck Fluoro tends to measure higher values compared to this HPLC.

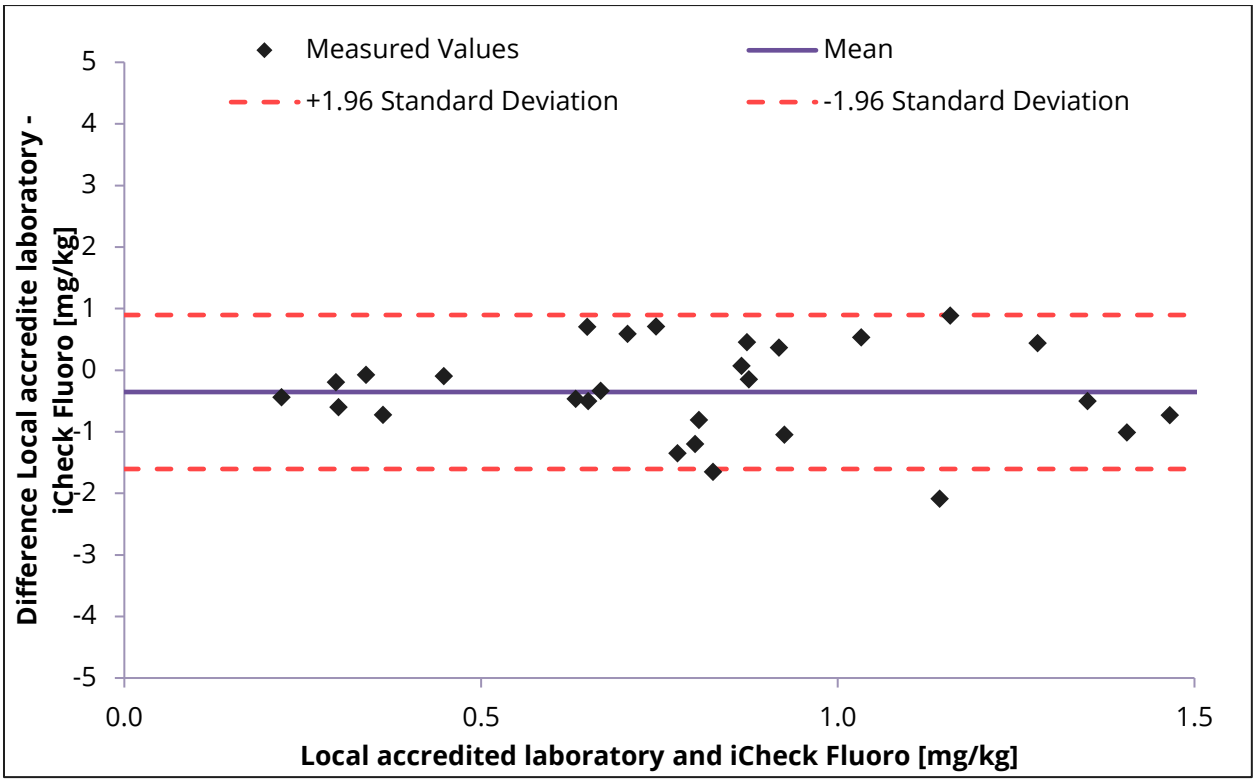


Figure 17: Comparison of the iCheck Fluoro vs. local accredited laboratory (HPLC) reference method for vitamin A in wheat and maize flour.

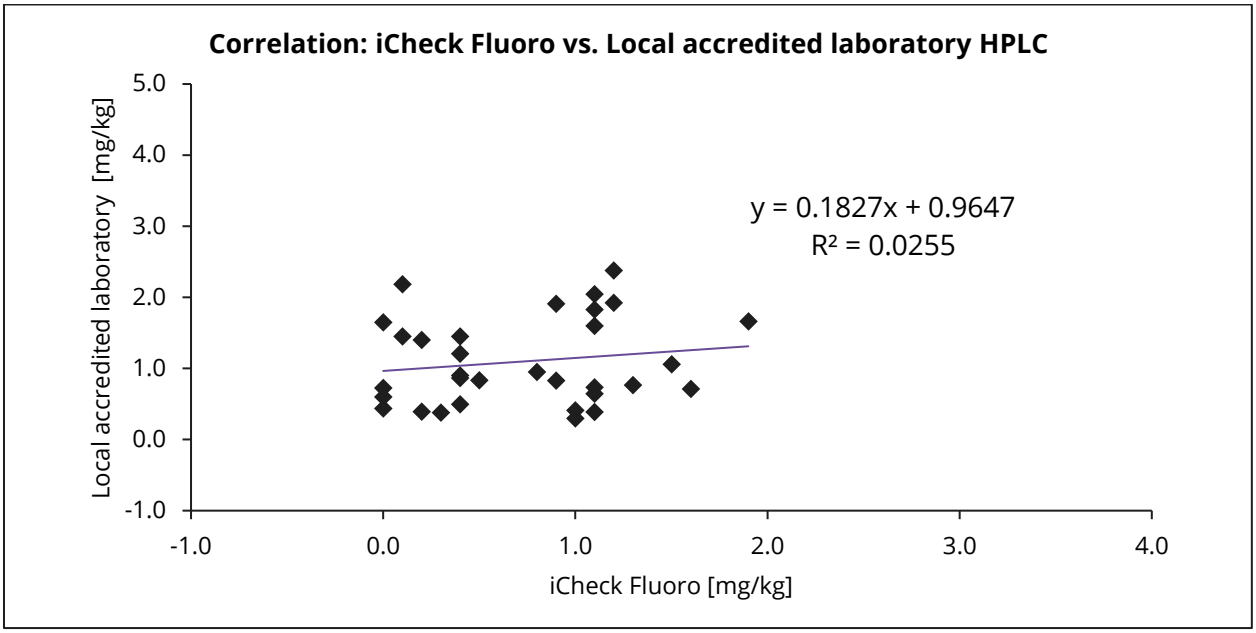


Figure 18: Linear correlation between the Vitamin A measurements from iCheck Fluoro and the Local accredited laboratory (HPLC) method.

Correlation analysis of iCheck Fluoro with HPLC at local accredited laboratory also is quite poor. The recovery of spiked samples at local laboratory was quite variable it requires further

investigation to have a good assessment of recovery and variation before any further interpretation.

Method Comparison – iCheck vs. External laboratory HPLC in Germany

The following illustration presents a comparative analysis of vitamin A measurements in wheat and maize flour samples with iCheck Fluoro vs. an external laboratory in Germany (HPLC). HPLC methods yielded a higher percentage of samples that measured very low levels of Vitamin A (63%, n=20).

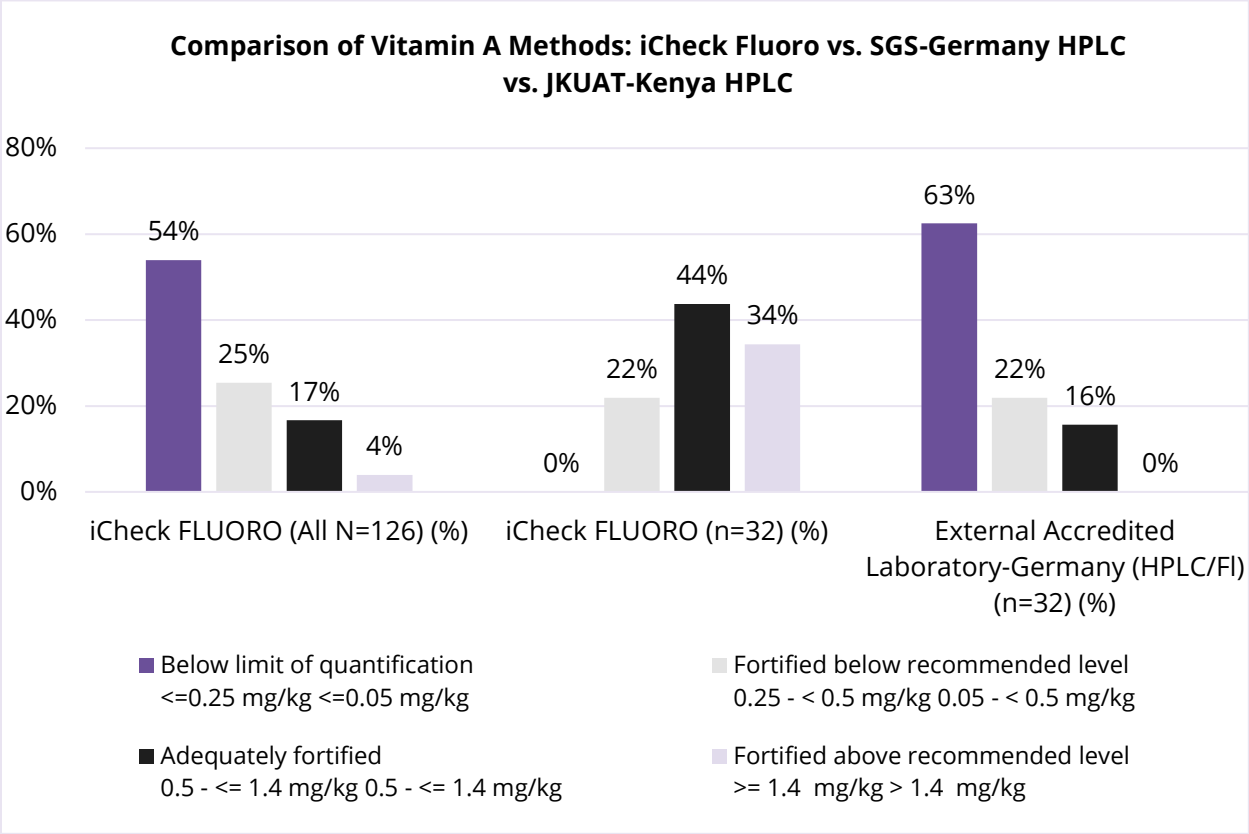


Figure 19: Comparison of Vitamin A levels as assessed with different methods: iCheck Fluoro for all samples (n=126) and same samples as analyzed with HPLC (n=32) vs. external laboratory-Germany HPLC (n=32)

Vitamin A results obtained with the external laboratory HPLC method and iCheck Fluoro were further compared. Bland-Altman analysis shows a mean difference of -0.78 mg/kg, indicating a bias where iCheck Fluoro tends to measure higher values compared to the external laboratory HPLC results. While correlation yields Pearson coefficient 0.55 also demonstrating poor correlation.

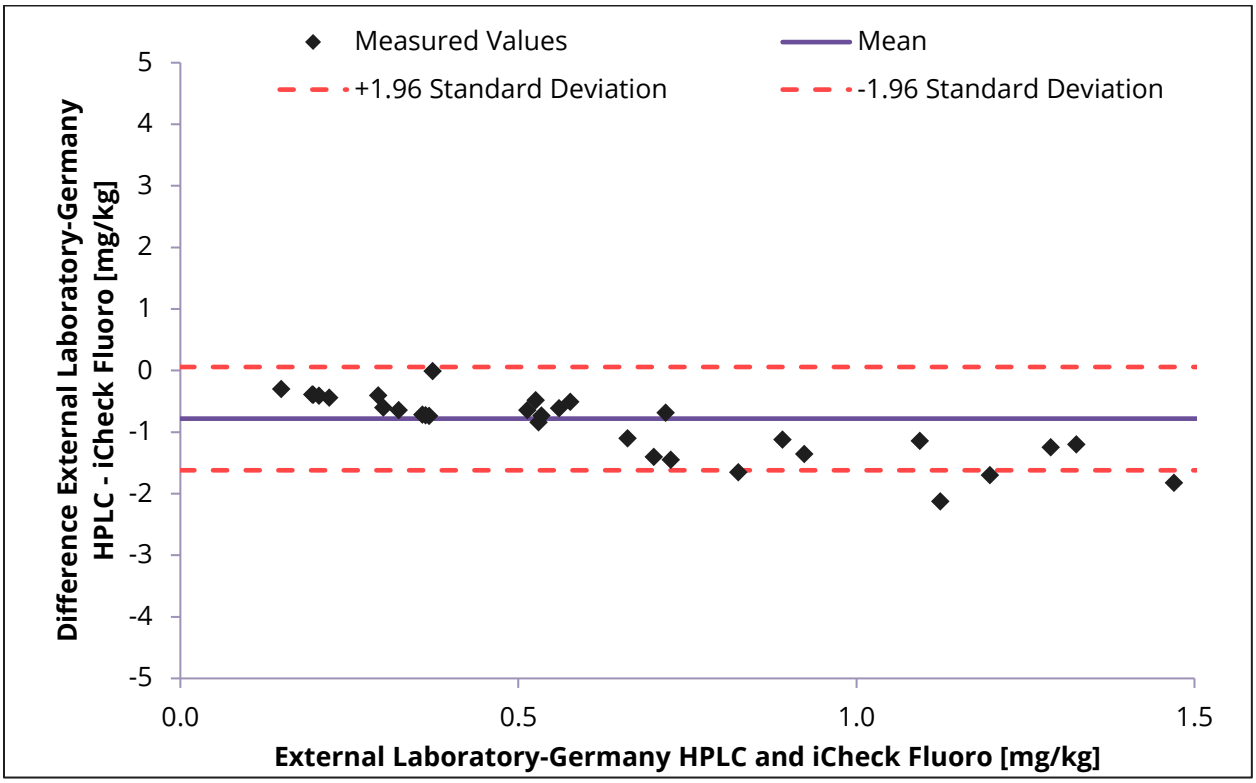


Figure 20: Comparison of the iCheck Fluoro vs. External Laboratory-Germany (HPLC) reference method for measuring vitamin A in wheat and maize flour samples.

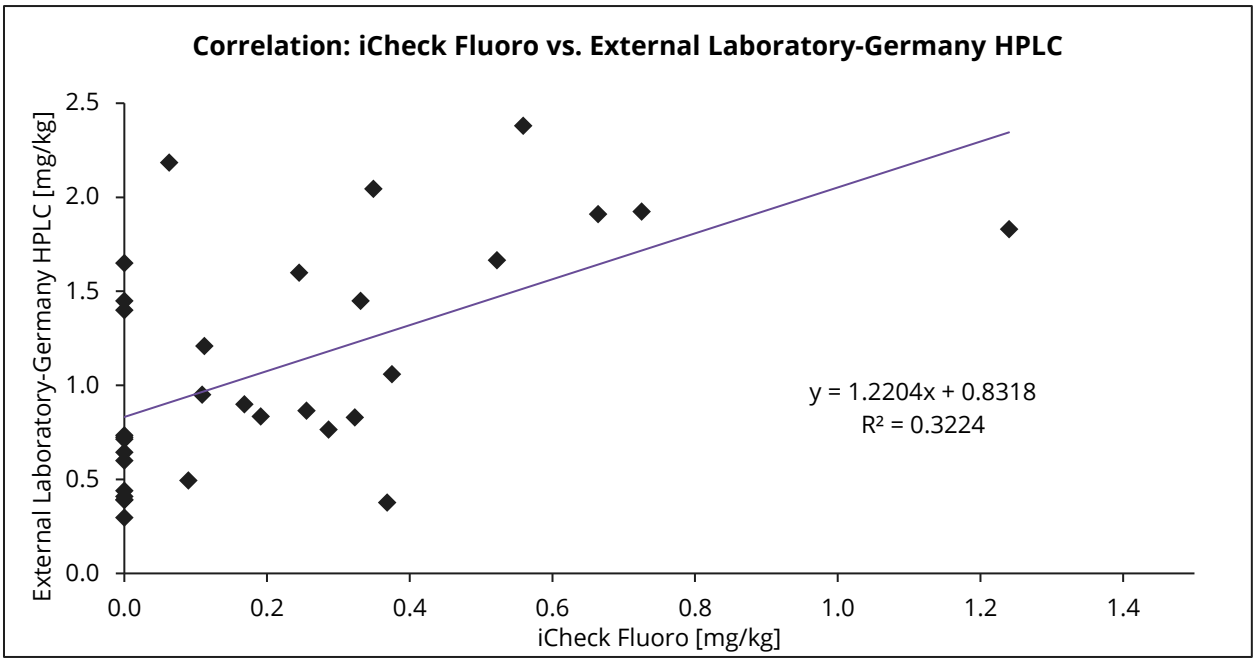


Figure 21: Linear correlation between the Vitamin A measurements from iCheck Fluoro and External Laboratory-Germany (HPLC) reference method for measuring vitamin A in wheat and maize flour samples.

Then vitamin A results obtained by the two accredited laboratories were further compared. Bland-Altman analysis shows a mean difference of -0.57 mg/kg, indicating a small bias where measures obtained at the local accredited laboratory in Kenya tends to measure higher

values compared to external laboratory–Germany HPLC results. While correlation yields Pearson coefficient 0.50 also demonstrating poor correlation.

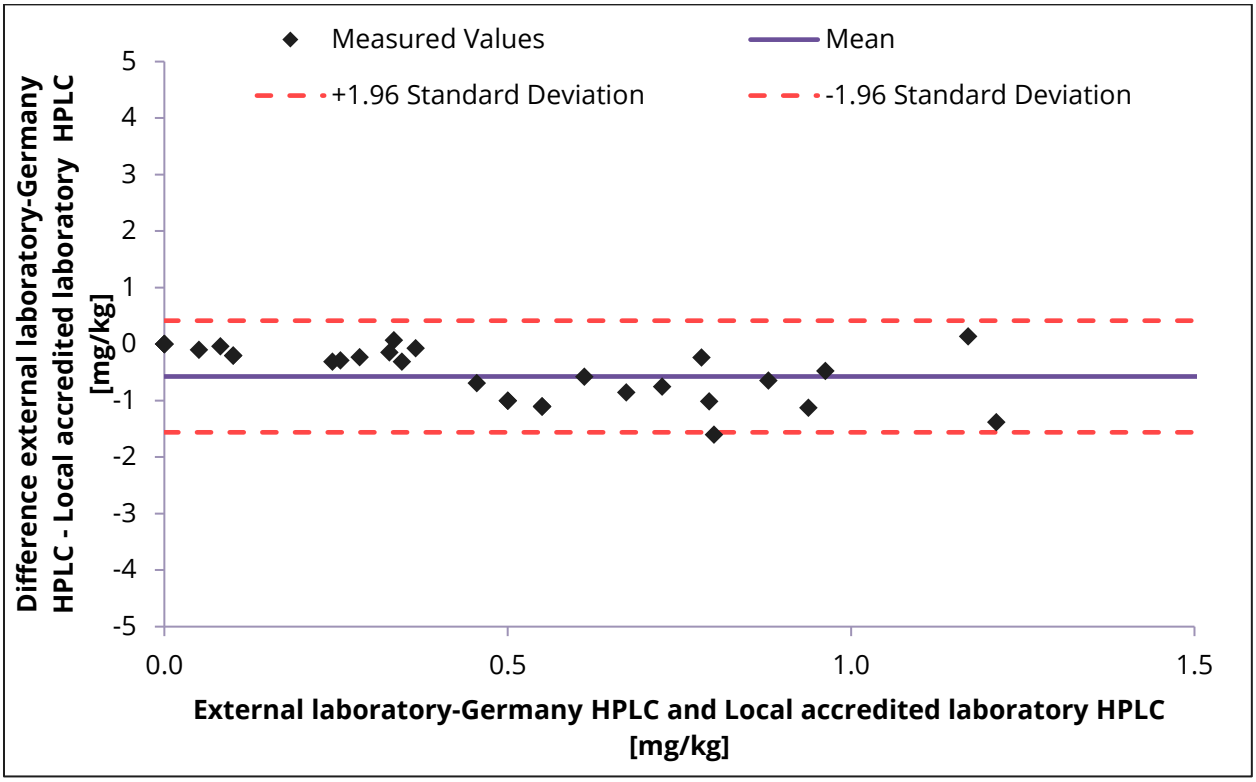


Figure 22: Comparison of the external laboratory-Germany vs Local accredited laboratory (HPLC) reference method for measuring vitamin A in wheat and maize flour samples.

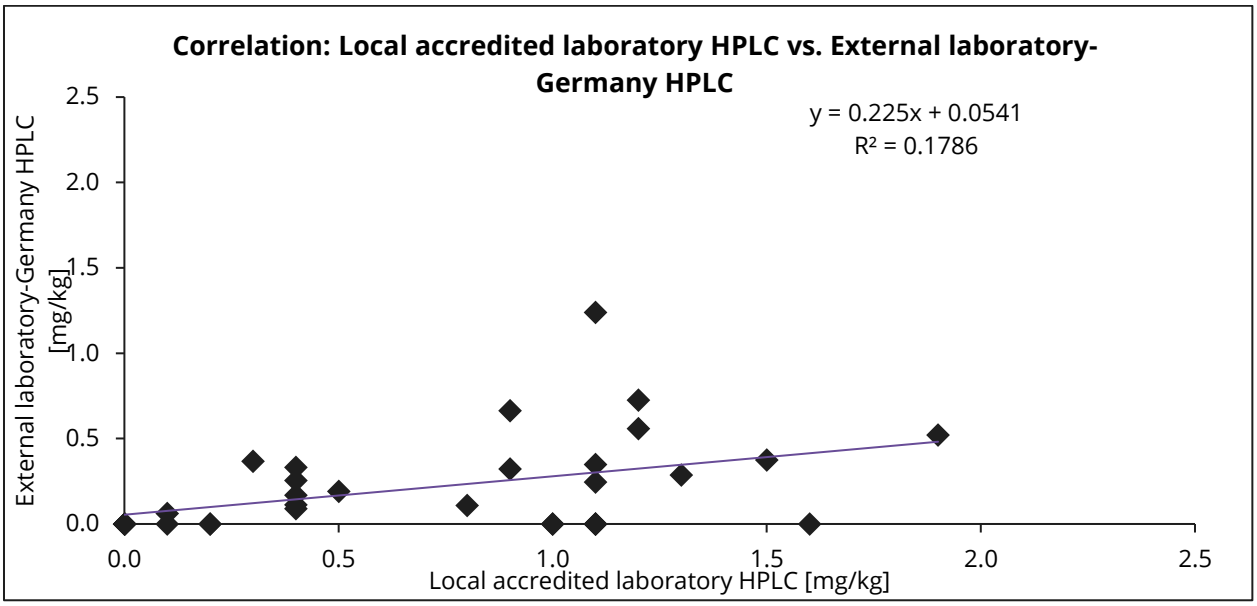


Figure 23: Correlation of vitamin A results between external laboratory-Germany & Local accredited laboratory (HPLC) reference method in wheat and maize flour samples.

Overall, the correlation of results for vitamin A in flour across all three data sets is consistently poor. The assessment of recovery of vitamin A in spiked control wheat flour sample was done with iCheck Fluoro and with Local accredited laboratory HPLC yielding 0.4-1.3 mg/kg and 0.8-1.4 mg/kg respectively with a sample fortified to an expected level of 1.4 mg/kg. This recovery is reasonable considering the very low vitamin A levels and sample homogeneity (it is very difficult to have a homogenous fortified flour sample).

Determination of Iron in wheat and maize samples

When determining iron in wheat and maize samples using the qualitative iron spot test, in wheat flour, 82% (n=67) of the samples tested positive for iron, while in 17% (n=14) no added iron was detected. The results for maize flour show a lower detection rate, with 68% (n=25) of the samples testing positive for iron and 32% (n=12) testing negative.

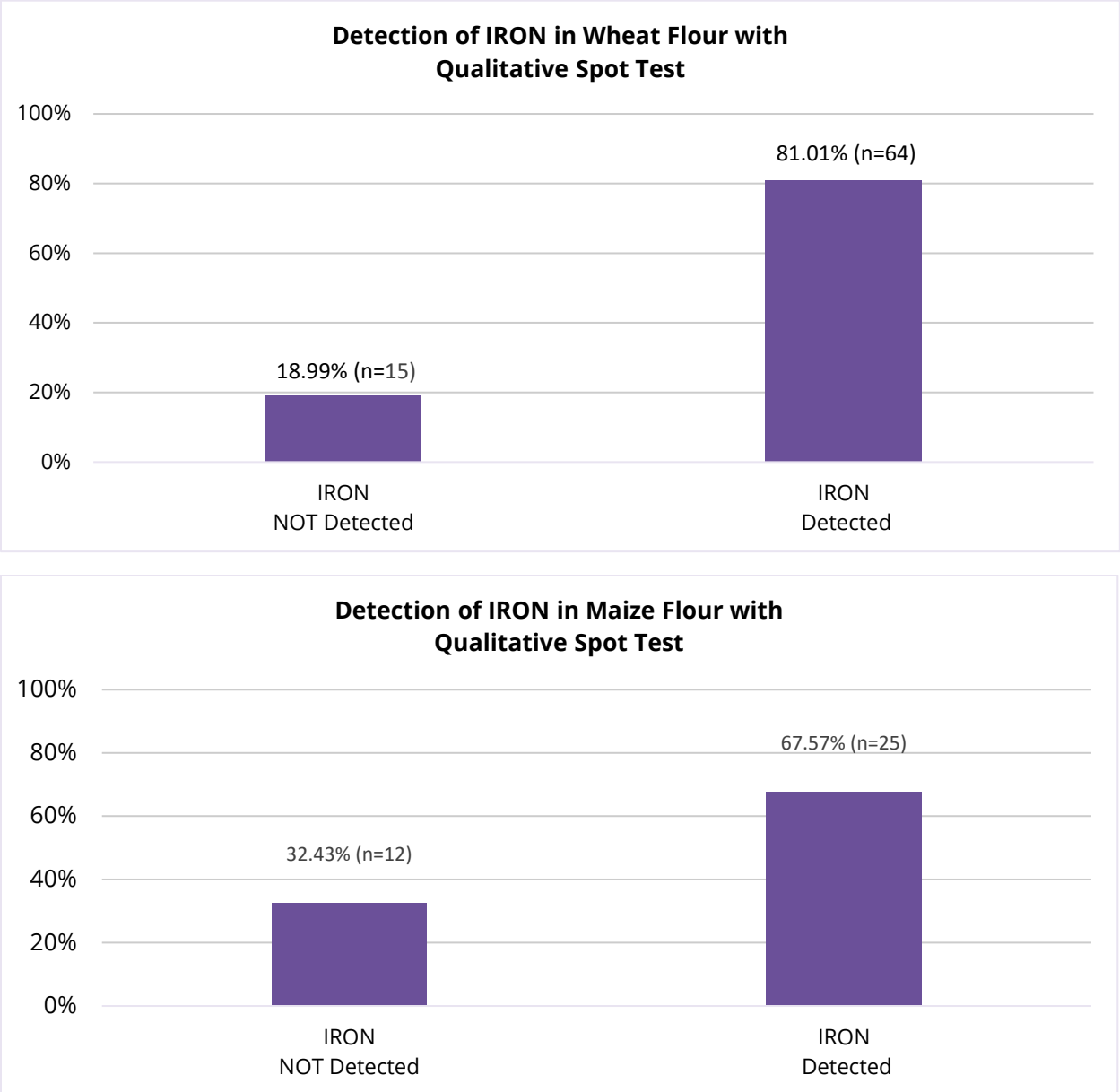


Figure 24: Qualitative spot test of iron in wheat (n=81) and maize flour (n=39).

Moreover, the specific use of chemical colorimetric reactions in this method enabled the qualitative iron spot test to provide insights into the types of iron compounds detected in wheat and maize flour samples. The detection of iron in wheat flour samples revealed that 89% of the samples contained iron fumarate, indicating it as the most prevalent form of iron fortification in these samples. Additionally, 11% of the wheat flour samples contained IRON NaFeEDTA, while 14% had a combination of iron fumarate and NaFeEDTA. In contrast, the maize flour samples showed a different distribution of iron compounds. The majority of the maize flour samples, 77%, contained IRON NaFeEDTA, while 15% contained iron fumarate.

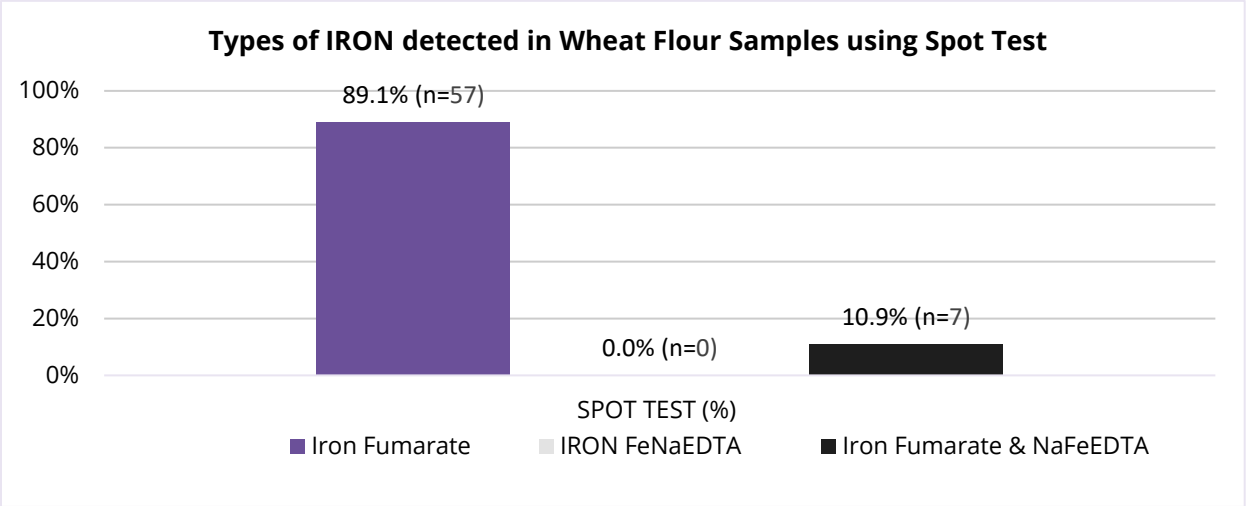


Figure 25: Distribution of the types of iron detected in wheat (n=64 – Iron detected) samples using a qualitative iron spot test method.

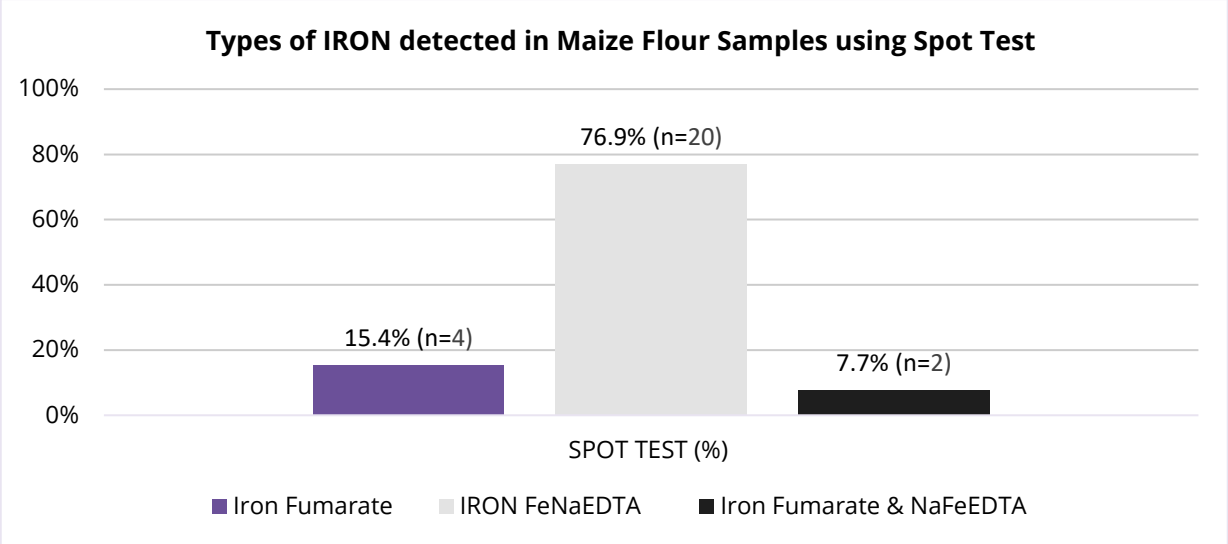


Figure 26: Distribution of the types of iron detected in maize (n=25 – Iron detected) samples using a qualitative iron spot test method.

Kenya has a minimal fortification national standard for total iron in wheat flour of 20 mg/kg, and 21 mg/kg in maize flour. Requirements for micronutrients in fortified milled wheat and maize products allow only NaFeEDTA, 12.5 % Fe, min or Ferrous fumarate, 32 %, min to be used as a source of iron to be added. Considering that iCheck Iron linear range is 1.5-12.0

mg/L (15-120 mg/kg) and the dilution factor of 10. Results with iCheck were grouped in the following way:

- Below limit of quantitation: <= 15 mg/kg
- Fortified below recommended level: >15 - < 20 mg/kg – for wheat
- Fortified below recommended level: >15 - < 21 mg/kg – for maize
- Adequately fortified: >20 mg/kg – for wheat
- Adequately fortified: >21 mg/kg – for maize

The following figure illustrates the distribution of iron fortification levels in wheat and maize flour samples as measured by the iCheck Iron device. In the case of wheat flour, a majority of the samples, 86% (n=70), are adequately fortified with iron, meeting or exceeding the recommended level of 20 mg/kg. Conversely, 8% of the wheat flour samples were found to be fortified below the recommended level, ranging from 15 to less than 20 mg/kg. only 2% were below 15 mg/kg.

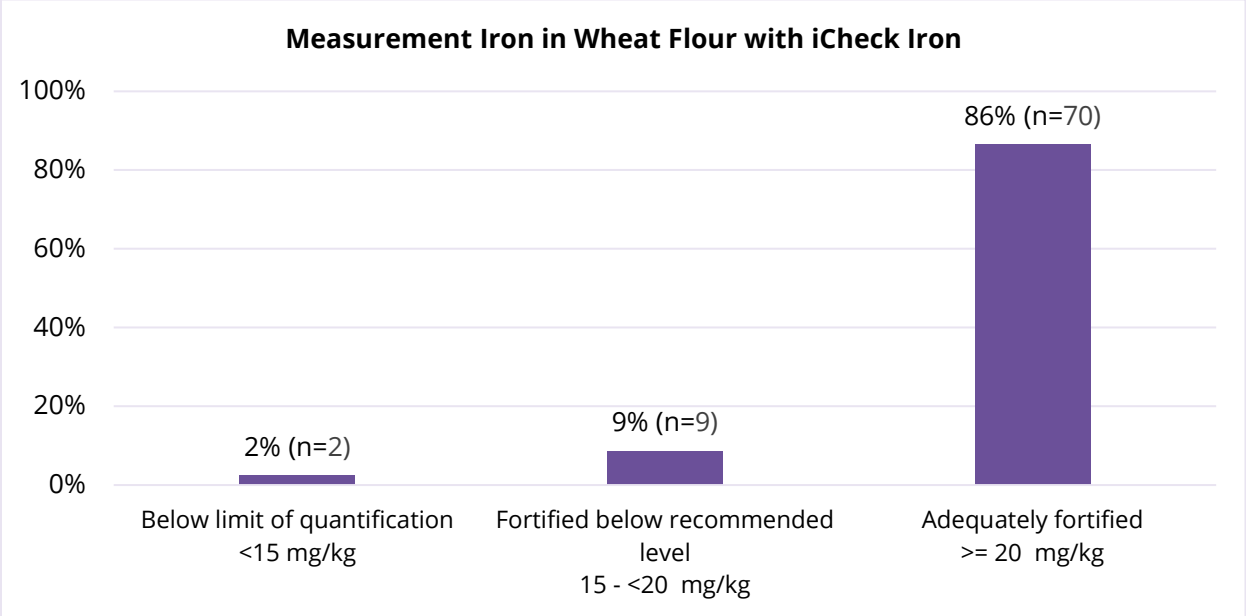


Figure 27: Measurement Iron in wheat (n=81) flour with iCheck Iron.

The difference in the total number of samples tested with iCheck Fluoro and qualitative vitamin A test are due to sampling handling and reporting generated by the trained local analysts. Only samples with a measured value using iCheck were used in the above chart. The results for maize flour similarly show a high level of compliance with fortification standards. Specifically, 62% (n=24) of maize flour samples are adequately fortified with iron, exceeding 21 mg/kg.

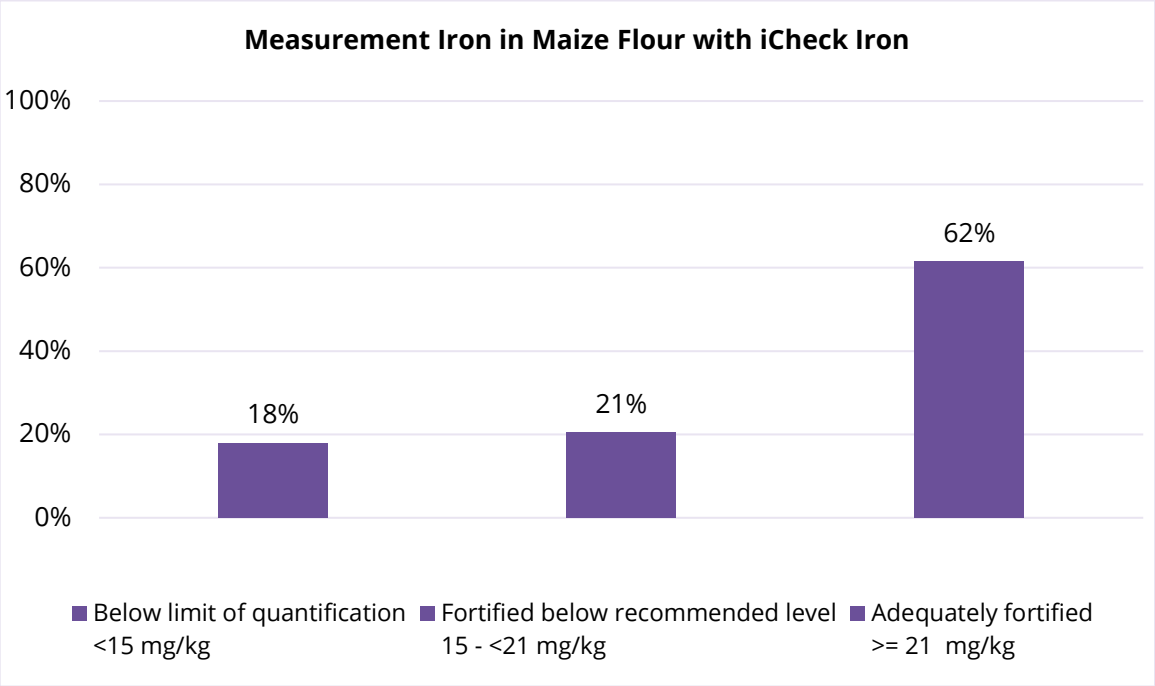


Figure 28: Measurement Iron in maize (n=39) flour with iCheck Iron.

Method Comparison – iCheck vs. Local accredited laboratory-Kenya

The following comparative analysis of iron measurements in wheat and maize flour samples using two different quantitative methods: iCheck Iron vs. Local accredited laboratory AAS, enables to contrast results obtained in samples analyzed with all these methods. From this scope, results are more comparable for iron than with vitamin A. Interestingly, the distribution of samples is highly comparable between iCheck Iron and Local accredited laboratory AAS, with 78%(n=25) and 66% (n=21), respectively.

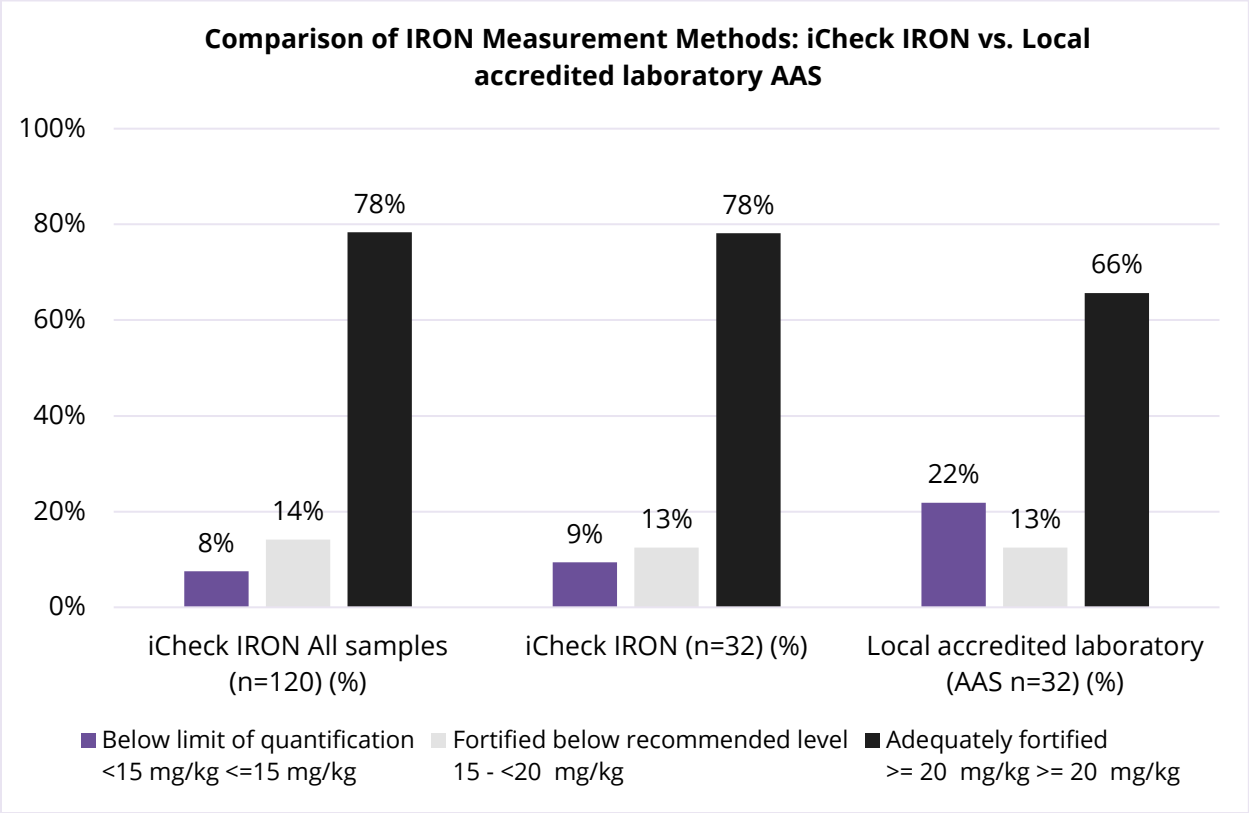


Figure 29: Comparison of the distribution of measurements of Iron in different detection methods: iCheck Iron (all and n=120) vs. Local accredited laboratory AAS (n=32). Only samples that were tested in all methods were considered for this comparison. Both maize and wheat samples were included.

However, the comparison of methods using a Bland-Altman analysis showed some interesting differences when considering the actual values that each sample obtained in both methods. Here, a mean difference of -11,18 mg/kg, indicating a bias where iCheck Iron tends to measure lower values compared to local accredited laboratory AAS.

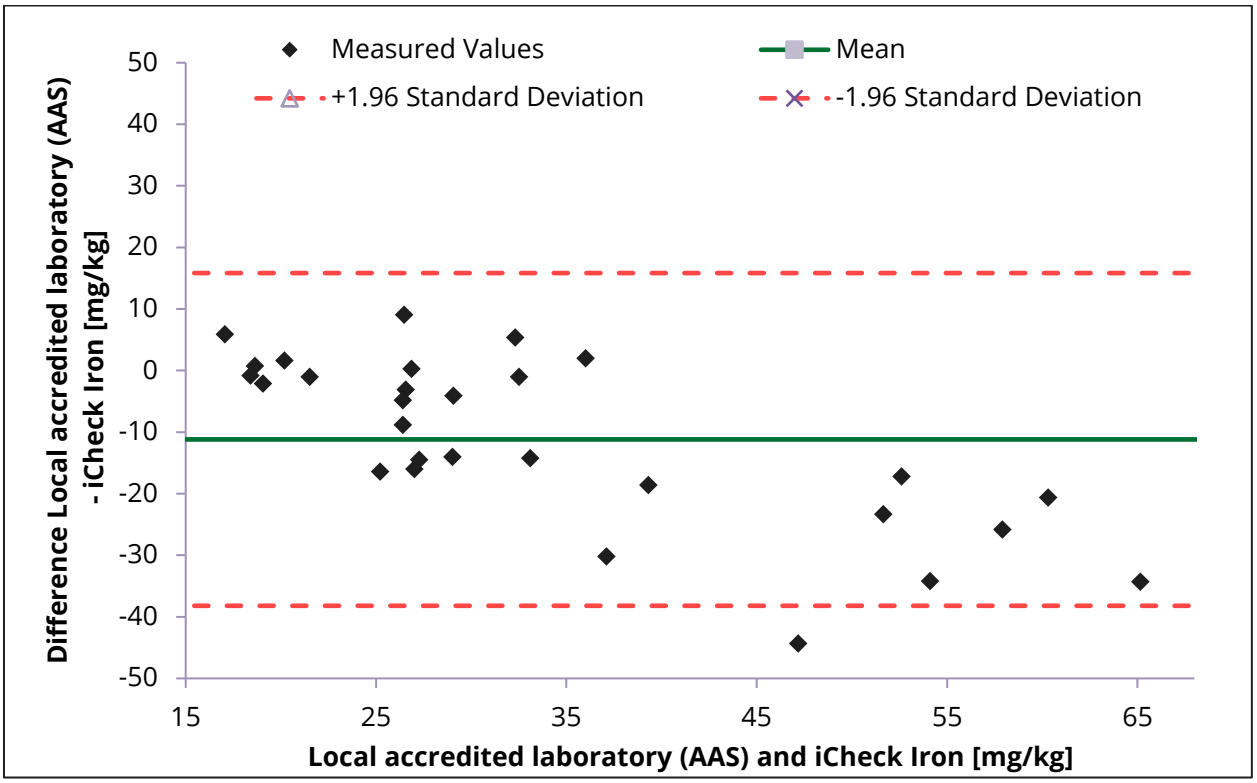


Figure 30: Comparison of the iCheck Iron vs. local accredited laboratory (AAS) reference method.

Additionally, the correlation yields Pearson coefficient of 0.76 indicate a relatively good correlation considering sample homogeneity.

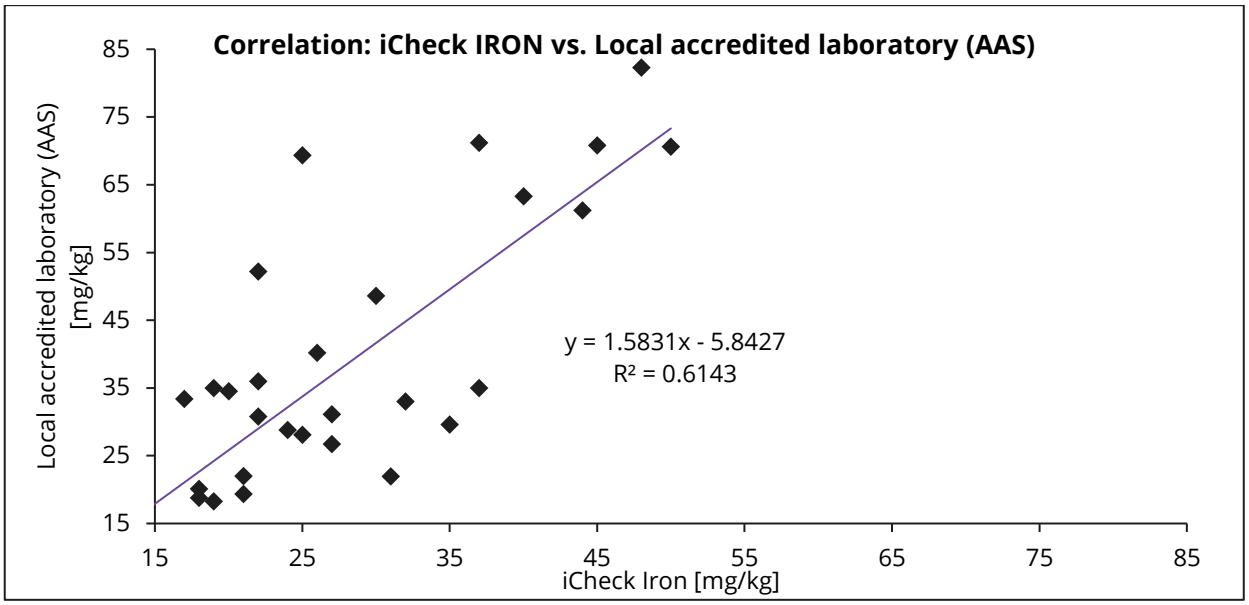


Figure 31: Linear correlation between the iron measurements from iCheck Iron and Local accredited laboratory (AAS) method.

Method Comparison – iCheck vs. External laboratory-Germany

To understand if the previous correlation values, fit the range of the correlation between iCheck and external laboratory-Germany, the following comparative analysis of iron measurements in wheat and maize flour samples were quantified:

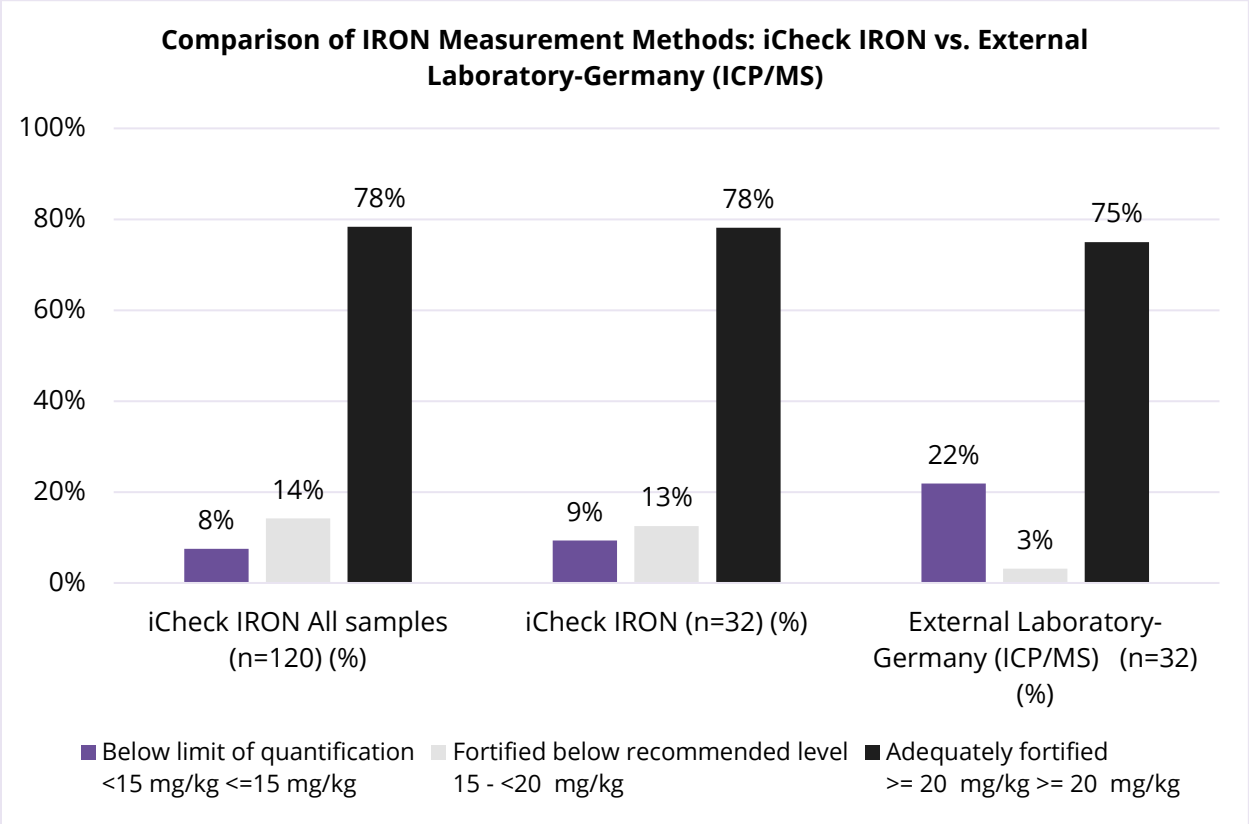


Figure 32: Comparison of the distribution of measurements of Iron in different detection methods: iCheck Iron (all and n=120) vs. External laboratory-Germany ICP (n=32) Only samples that were tested in all methods were considered for this comparison. Both maize and wheat samples were included.

Iron results obtained with the external laboratory-Germany ICP method and iCheck Iron were further compared. Bland-Altman analysis shows a mean difference of -3.32 mg/kg, indicating a bias where iCheck Iron tends to measure higher values compared to external laboratory ICP. While correlation yields Pearson coefficient 0.82 also demonstrating strong correlation.

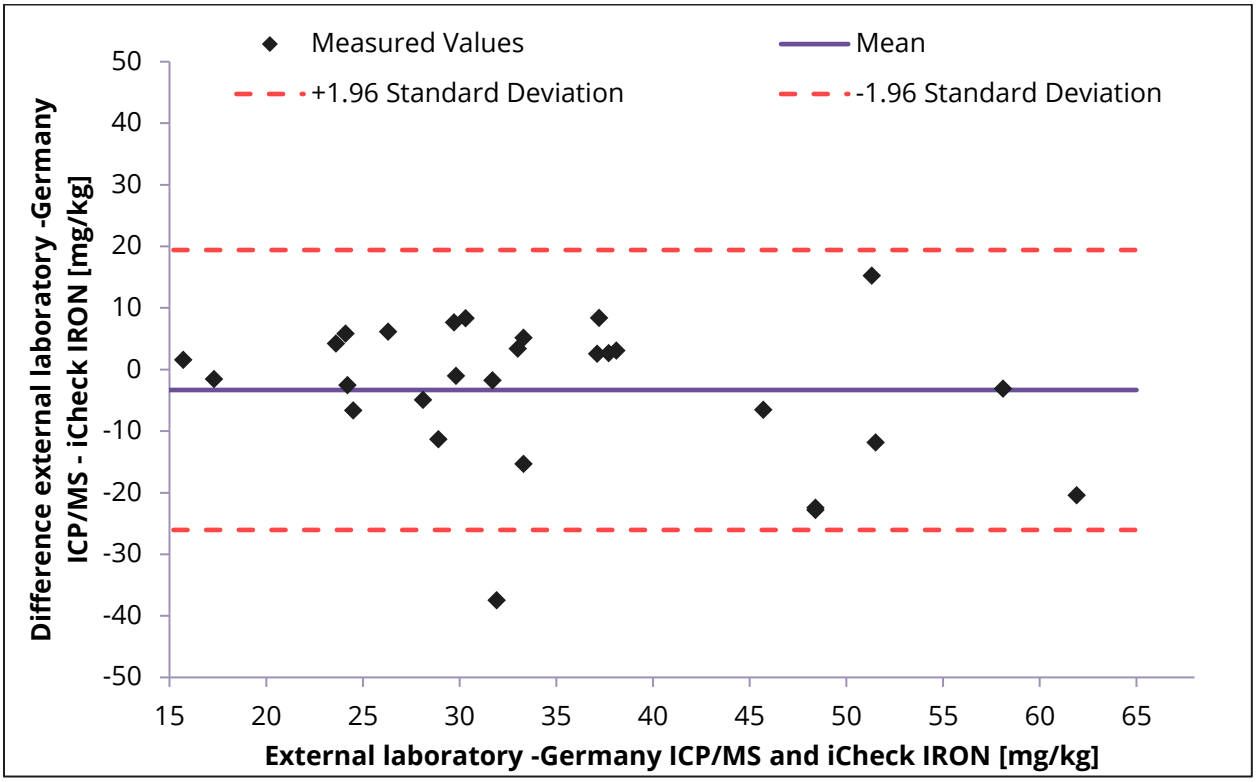


Figure 33: Comparison of the iCheck Iron vs. External laboratory-Germany (ICP/MS) reference method.

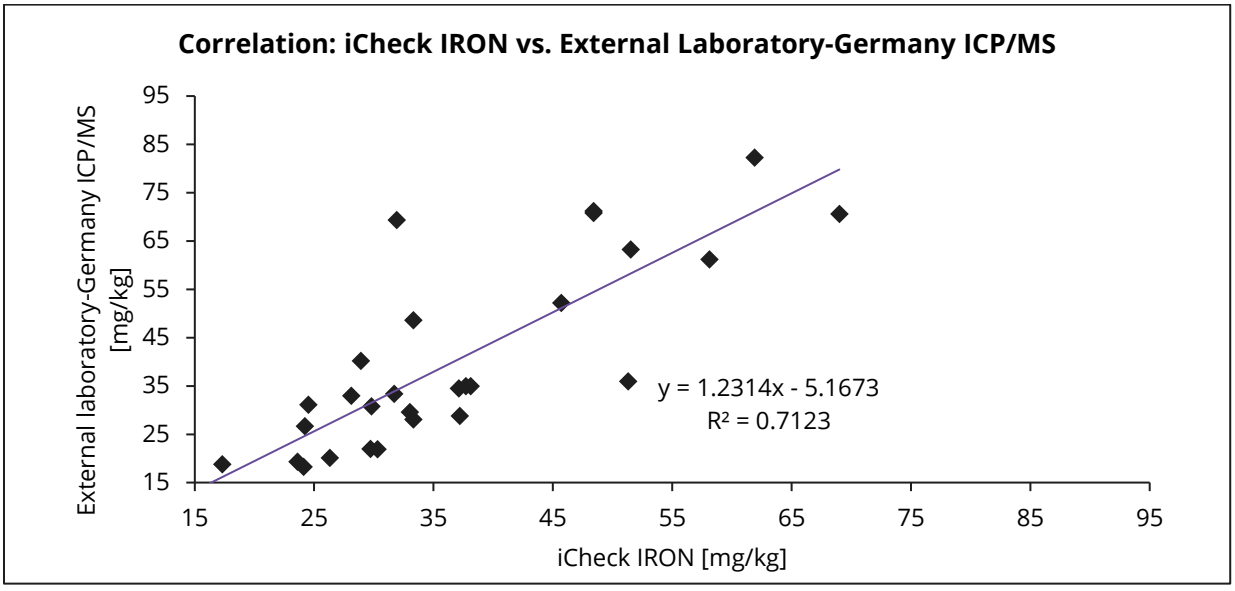


Figure 34: Linear correlation between the iron measurements from iCheck Iron and external laboratory - Germany (ICP/MS) method.

Finally, iron results obtained with the local accredited laboratory AAS method and external laboratory ICP were further compared. Bland-Altman analysis shows a mean difference of -11.18 mg/kg, indicating a bias where iCheck Iron tends to measure higher values compared to the local accredited laboratory AAS method. While correlation yields Pearson coefficient

0.76 also demonstrating weaker correlation than with iCheck Iron and external laboratory results.

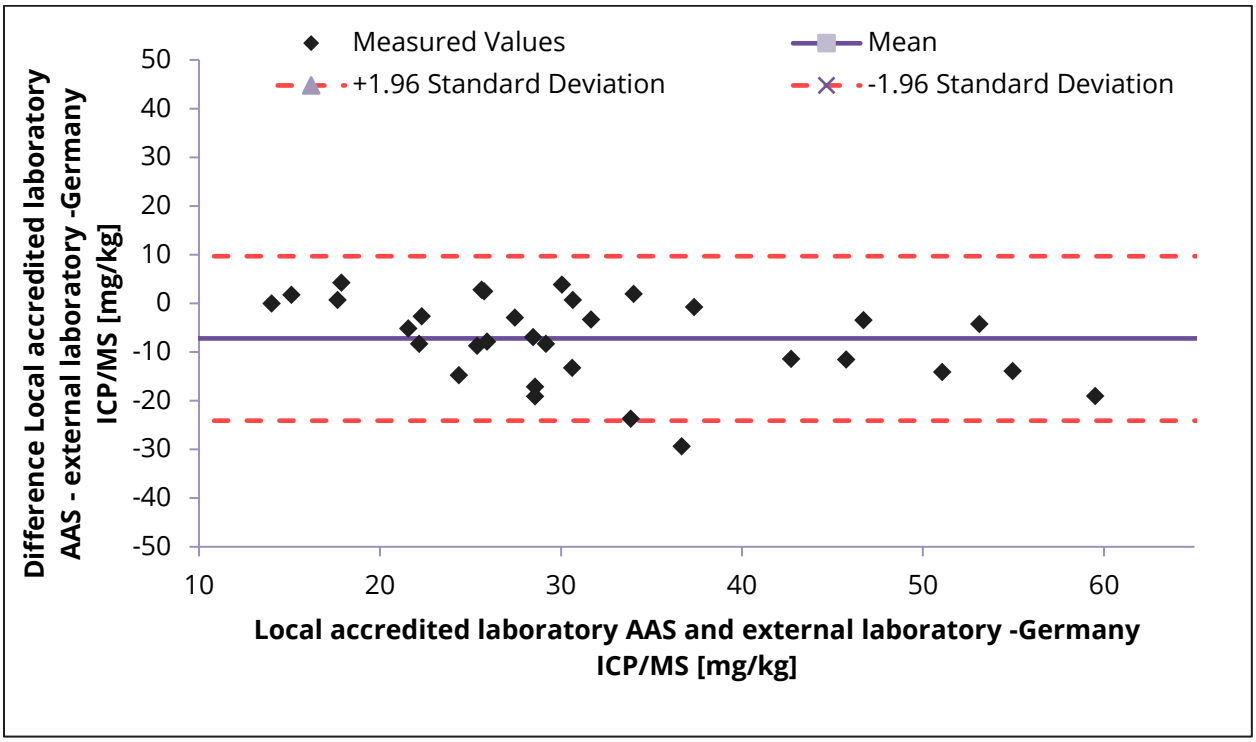


Figure 35: Comparison of External laboratory-Germany (ICP/MS) reference method vs. local accredited laboratory (AAS) reference method.

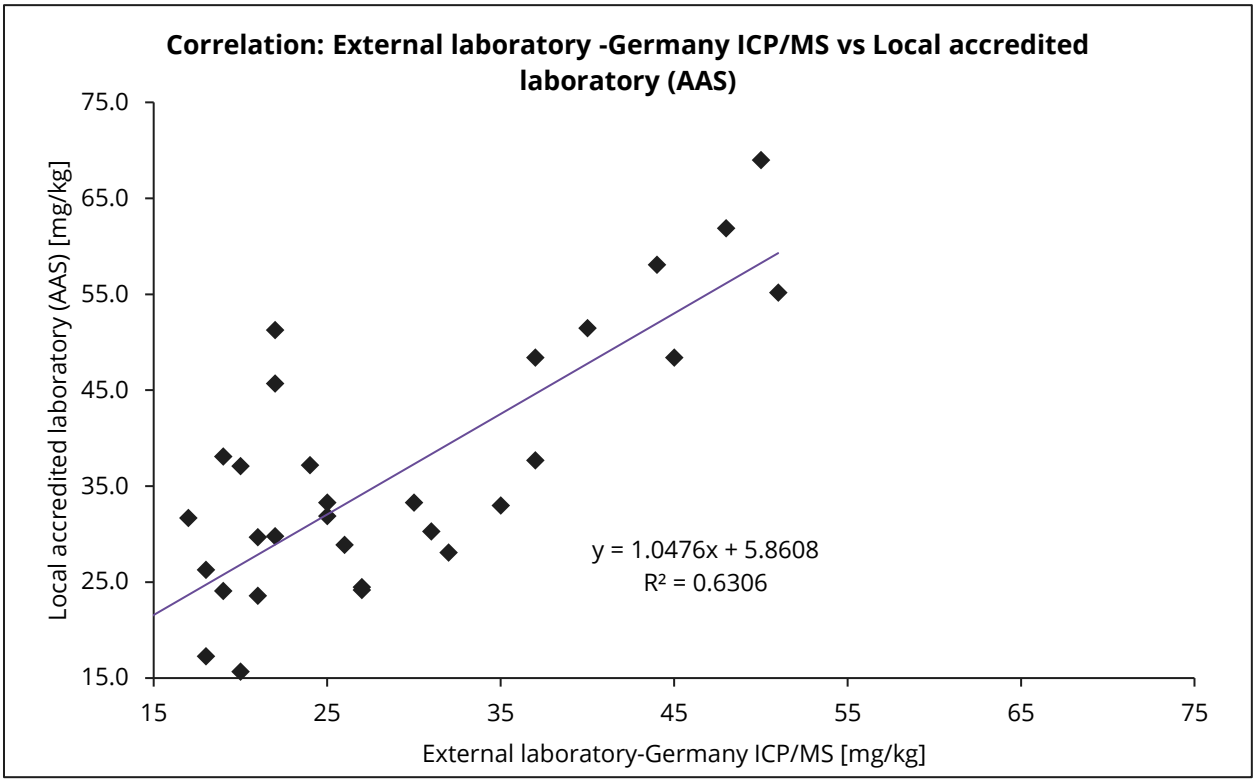


Figure 36: Linear correlation between the iron measurements from external laboratory -Germany ICP/MS vs. the Local accredited laboratory (AAS) reference method.

The correlation of results for iron in flour across all three data sets is quite good. The assessment of recovery of iron (NaFeEDTA) in spiked control wheat flour sample was done with iCheck Iron and with Local accredited laboratory AAS yielding 22-35 mg/kg and 19-21 mg/kg respectively with a sample fortified to an expected level of added iron of 30 mg/kg plus <10 mg/kg intrinsic iron. This recovery is reasonable when considering sample homogeneity (it is very difficult to have a homogenous fortified flour sample).

KENYA: CHALLENGES AND RECOMMENDATION DURING PLANNING AND IMPLEMENTATION

Challenges	Impact on Asssesment	Control Measures & Mitigation	Recommendations
Samples could only be sourced from already ongoing studies by TechnoServe. This posed challenges aligning timing of sampling and testing	How representative were the samples of the market; Stability of vitamin A	No control measured where needed regarding market representation as TechnoServe samples covered that. A freezer was procured and samples were stored at AMS until testing.	Collection must be planned and aligned with the testing period for time sensitive samples such as fortified oil.
Issues with importation of iChecks and kits into the country. Several factors: Lack of experience to import/export via temporary license with local distributor in Kenya.	iChecks and Kits Shipment	The distributor can ship using CIP (to the airport and around), followed by invoicing for import costs and internal shipment. Importation via the normal process (full taxes), where the distributor purchases in advance at lower prices. Post-assessment, the devices remain with the distributor for internal sales.	Review if the distributor has an updated importation license. Consider different importation methods: 1. Transporting iChecks with analysts from Germany. 2. Direct sale to the distributor through normal importation. 3. Temporary import/export for demos and tradeshows.
Outsourcing of chemicals locally reported to be a time-consuming and difficult to coordinate process.	Qualitative testing preparation	Mitigation included procuring extras and utilizing help from local partners (AMS) for local sourcing of necessary chemicals.	Strengthen local partnerships to facilitate the sourcing of chemicals and streamline importation processes.

3. INDIA

BACKGROUND

India faces significant public health challenges related to vitamin A and iron deficiencies, which contribute to serious conditions such as anemia, impaired cognitive development, and increased morbidity and mortality. To address these deficiencies, the Indian government has implemented fortification programs for edible oil with vitamin A and rice with iron, both of which play crucial roles in improving public health outcomes.

Edible Oil Fortification:

The fortification of edible oil with vitamin A is a widespread intervention in India, given the high consumption of oil in Indian diets. The Food Safety and Standards Authority of India (FSSAI) mandates that vegetable oil, when fortified, must contain 6-9.9 µg RE per gram of oil, using retinyl acetate or retinyl palmitate. This initiative ensures that a broad population segment, including children and women, receives their required intake of vitamin A.

Among several organizations involved in promoting fortification of staple foods in India, the Global Alliance for Improved Nutrition (GAIN) has been actively involved in promoting oil fortification. GAIN supports the development and implementation of standards and provides technical assistance to oil producers to ensure compliance with fortification guidelines. Their efforts include policy advocacy, quality assessment of fortified staples, and capacity building for edible oil industries. Their participation in this project involved the mapping and sample collection of fortified edible oil.

Rice Fortification:

Iron deficiency anemia is a major health concern in India, particularly affecting women and children. Fortifying rice, a staple food consumed widely across the country, with iron is an effective strategy to combat this deficiency. Rice fortification may be considered as having the highest potential to fill the gap in current staple food fortification programs as it is the staple food of 65% of the Indian population and reaches the most vulnerable and poorer

section. The process involves the use of fortified reconstituted kernels (FRK), which are rice-shaped extruded rice-alike products enriched with iron, folic acid, and other micronutrients, which are then blended with regular rice to improve nutritional content. Typically, fortified kernels are mixed with regular rice in a 1:100 ratio (1 grain of FRK per each 99 rice grains).

The Food Safety and Standards Authority India set out the specifications for fortified rice must include specific levels of essential micronutrients to address nutritional deficiencies. The mandatory micronutrient levels per kilogram of fortified rice are as follows:

- Iron: 28 mg to 42.5 mg (FSSAI.FRK.16.004.2023 or FSSAI.FRK.16.004.2023)
- Folic Acid: 75 µg to 125 µg (FSSAI.FRK.16.005.2023)
- Vitamin B12: 0.75 µg to 1.25 µg (FSSAI.FRK.16.006.2023)

Additionally, optional micronutrients that can be included are zinc, vitamin A, thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), and pyridoxine (vitamin B6). These FRK have a shelf life of at least 12 months and are distributed through various government schemes to ensure that vulnerable populations have access to nutritionally enhanced rice, significantly reducing anemia and improving cognitive and physical development.

PATH, an organization at the forefront of rice fortification in India, supports the production of high-quality, cost-effective FRK and works with state governments and private sectors to increase the number of FRK manufacturers and blenders, enhancing the affordability and availability of fortified rice. Their participation in this project was to collect samples directly from producers, since in order to obtain a testing samples that can allow for comparative purposes, the sample collection at the production site is imperative before blending with regular rice. Their intervention to connect directly with producers was crucial in order to obtain samples to be used for our analysis purposes.

SAMPLE COLLECTION

GAIN planned and organized the collection of edible oil samples from marketplaces, acting as a local partner. The market assessment methodology sampled retail outlets across three representative levels of the market in India, covering two states: Tamil Nadu (TN) and Madhya Pradesh (MP). The collection of samples took place between September 2023 and January 2024. This sampling is part of a larger separate market assessment study that GAIN is currently leading.

A total of 814 oil samples (including rice bran oil, peanut oil, oil blends, vegetable oil, sunflower oil, soybean oil, mustard oil, palm oil, and canola oil) were collected, with volumes between 250-500 mL and production dates as early as January 2023. GAIN labeled all samples in advance with unique codes. For this assessment, we selected 114 samples based on criteria such as the date of collection, production date, and different batches per brand. GAIN shipped all 814 samples directly to the testing facility in Delhi, which required an additional selection process to identify and select the requested sample codes.

Considering the sensitivity of Vitamin A to degradation over time in edible oils, we requested GAIN to ensure proper storage of samples to minimize risk factors affecting Vitamin A stability. Degradation may occur over time, especially with longer storage periods, due to factors like light exposure, oxidation agents, heat, and humidity. To ensure the accuracy and relevance of our study, we used samples produced from September 2023 onwards. After eliminating duplicates and outdated samples in favor of more recent ones, we selected a total of 103 samples.

Regarding fortified rice kernels (FRK), after reviewing data from FRK manufacturers with active licenses and supplier reports from India, we identified an 80% coverage of the FRK market. Notably, Punjab emerged as a critical state for FRK production and supply, contributing 40% to the total production capacity and housing 30% of FRK manufacturers and 36% of FRK suppliers in India. We suggest that sampling from FRK producers in states with the highest production capacities—Punjab, Haryana, Uttar Pradesh, West Bengal, Telangana, and Chhattisgarh—could guarantee an 85% market coverage.

However, as FRK samples are not available in the open market they had to be collected directly from manufacturers willing to take part in this study. Under these circumstances, it was not possible to collect samples representing 80% of the Indian FRK market. Along with IIT Delhi, PATH collected a total of 26 samples directly from manufacturers. The date of sample collection is undetermined, as some samples might have been collected before the start of this project. PATH handled sample storage and shipped the collected samples to the testing facility in Delhi before testing began.

TRAINING METHODOLOGY

Before the testing of samples, it was essential to train local analysts in analytical methods. This training aimed to ensure that, during the on-site phase, the newly trained analysts could conduct sample testing independently with minimal oversight. Dr. Santiago Andrade and Dr. Anna Zhenchuk from BioAnalyt conducted this training, focusing on two key analysts from the hosting facility, Avon Food Labs, in Delhi, India.

Following the introduction of the project with local stakeholders and partners involved in the coordination of this onsite, the training of the methodologies to be used was provided to two analysts from this facility. The training methods included both qualitative colorimetry testing for Vitamin A in edible oil and Iron in FRK, and quantitative testing using the iCheck Choma 3 and iCheck Iron.

At the beginning of the session, the trainers provided a comprehensive guide on sample handling procedures and described the protocols for qualitative colorimetric testing, including the preparation of the necessary solutions. Following this, an in-depth introduction to the iCheck Chroma 3 and iCheck Iron was given, covering step-by-step calibration of the device, sample preparation, readout, and interpretation. Detailed instructions for each technique are outlined in the following sections.

TESTING METHODOLOGY

The intended study in India included the measurements of fortification levels of Vitamin A in edible oil and Iron in FRK. The testing took place at Avon Food Labs in Delhi.

The testing methodology for the analysis of vitamin A in edible oil and Iron FRK samples began with qualitative colorimetric testing. This colorimetric test allowed analysts to provide a preliminary assessment of the presence of vitamin A or Iron, respectively. The readout of this experiment resulted in a Yes (fortified) /No (not fortified) decision based on the visual evaluation of the reaction.

Subsequently, a quantitative analysis using the iCheck Chroma3 device was conducted to obtain precise measurements of the vitamin A content. iCheck Iron device was used to obtain measurements of Iron content in FRK samples. To ensure quality control, spiked control samples of edible oil and FRK of known iron concentration were analyzed after every tenth sample, and every tenth sample was analyzed in duplicates using the same protocol.

Finally, approximately 20% of the analyzed samples were sub aliquoted for further verification using reference methodologies in accredited laboratories. BioAnalyt/QuImpact trainers took oil samples for testing with HPLC at External laboratory in Germany, and FRK for testing with ICP/MS at an external laboratory in Germany. In this case, the samples selected for analysis in external laboratories were the same as those tested in a local accredited laboratory with HPLC, for edible oil samples, and AAS, for FRK samples.

QUALITATIVE TESTING: Colorimetric Assay

Analysis of Vitamin A with qualitative ring test

Colorimetric testing is widely used for screening the presence of vitamin A edible oil. In India, the regulatory authority FSSAI and the Bureau of Standard provide a method for quantification of Vitamin A in Vanaspati using an antimony trichloride method where the appearance of blue coloration indicates the presence of Vitamin A. The protocol preparation for this method is as follows:

Solution Preparation
Dissolve antimony trichloride in chloroform and store into an amber Schott Flask.

	Chemical	Number of Sample						Units
		2	10	20	100	150	200	
Solution 1	Antimony Trichloride	1,1	5,5	11	55	82,5	110	g
	Chloroform	2	25	50	250	375	500	mL

The use and handling of these chemicals require protective gloves and goggles to prevent severe skin irritation. These solutions remain stable for approximately 36 hours at room temperature.

Solution Preparation

- Step 1**:** Add 2.5ml of Solution 1 to a glass transparent test tube.
- Step 2**:** Add 5ml of the edible oil sample slowly while tilting the test tube. Avoid shaking the sample.
- Step 3**:** Observe a Blue Ring at the interface for the presence of Vitamin A in the sample of Edible Oil. This is a qualitative test only, detecting if Vitamin A is present or not.

It is recommended to use a glass transparent test tube to be able to observe color the sudden change of color in the ring formed at the interphase between oil and reagent:

Figure 37: Qualitative Analysis of the Presence of Vitamin A in edible oil using the ring test.

Analysis of IRON with qualitative assay

Solution Preparation

- Solution 1**:** Prepare a 2-N Hydrochloric acid (HCl) with distilled or bottled water.
- Solution 2**:** Prepare 10% Potassium Thiocyanate (KSCN) in distilled or bottled water. Potassium thiocyanate detects iron ions by forming a red iron-thiocyanate complex with iron ions (Fe^{3+}), allowing for visual detection.

The use and handling of these chemicals require protective gloves and goggles to prevent severe skin irritation.

Sample Analysis

- Step 1: Place at least 50 gr. of FRK or fortified rice in a plastic cup, tray or a similar container.
- Step 2**:** Pour the 2N HCl solution on the rice sample until all the rice is wet.
- Step 3**:** Pour a similar amount of 10% KSCN solution onto the rice sample.
- Step 4**:** Immediately, FRK will turn red to dark red (black upon drying) indicating the presence of iron. Reagents react with ferric (iron) ions to generate a dark brown-red pigment. This is a qualitative test only, detecting if iron is present or not.

Blank sample
No red kernels

IRON detected
In ground FRK

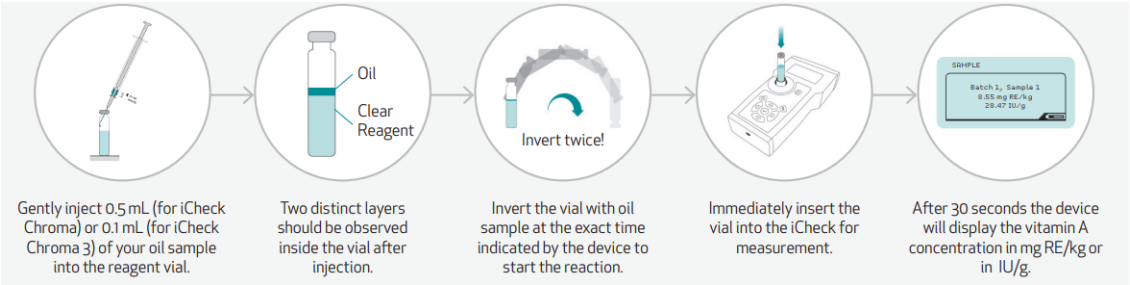
IRON detected
In whole FRK

Figure 38: Qualitative Analysis of the Presence of Iron in Fortified Rice Kernels.

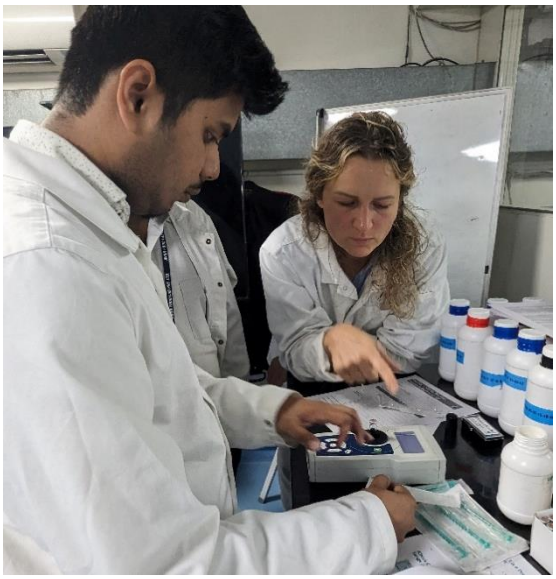
QUANTITATIVE TESTING: iCheck Chroma 3 for vitamin A in oil

The iCheck Chroma 3 is used for the determination of vitamin A in edible oil. This method is based on the Carr-Price reaction, where the reagents in the vial turn a brilliant blue in response to retinol, with the intensity proportional to the retinol concentration. The fundamental principle involves the reaction of retinol with antimony trichloride ($SbCl_3$) to generate a transient blue color. The iCheck Chroma 3 measures the absorption of this color at three different wavelengths over 30 seconds, calculating the vitamin A content through a sophisticated algorithm and displaying the result in mg retinol equivalents/kg of oil. The device has a linear range of 3–30 mg retinol equivalents (RE)/kg of oil.

The following is the sample preparation and analysis workflow for Vitamin A measurement:



A description of how to measure the sample is shown in the following training video: https://www.youtube.com/watch?v=s2Kyg90qyz0.



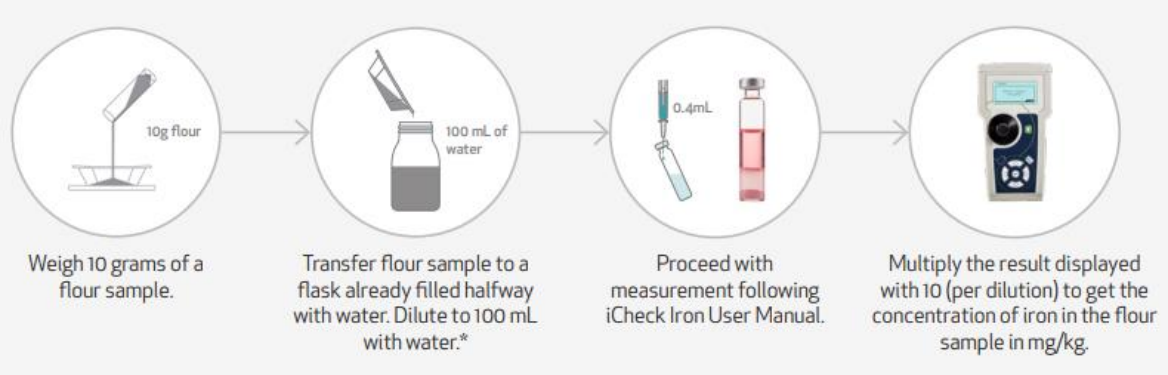
This method has been validated against reference methods in several publications. Most recently, a study compared a portable device to high-performance liquid chromatography (HPLC) in terms of quantification of vitamin A in both spiked and commercially fortified oils, taking measurements of nine different oil types (soybean, palm, cottonseed, rapeseed, corn, peanut, coconut, sunflower, and rice bran) spiked with retinyl palmitate at six different concentrations. Vitamin A recoveries were 97–132% for HPLC and 74–127% for iCheck Chroma 3, including a strong positive correlation, $r = 0.88$. Concluding that iCheck provides a lower-cost, quick, and user friendly alternative to HPLC with comparable performance (Palma Duran et. al, Food Anal. Methods, 2024).

QUANTITATIVE TESTING: iCheck IRON in FRK

The iCheck Iron is a portable, single-wavelength photometer, that quantitatively measures iron in multiple food matrices based on colorimetric detection. The iCheck Iron measures absorption at 525 nm, using reagents containing bathophenanthroline in organic solvent, along with reducing and chelating agents. The red color intensity correlates with the iron concentration. This intensity is measured by the device and converted to iron content, displayed in mg iron/kg of sample.

For sample preparation, the diluted sample is injected into a reagent vial prefilled with a chromogenic reagent, mixed, and then measured with the iCheck Iron device. The device measures color intensity at specific wavelengths for accurate iron quantification. Results are stored in the device and can be transferred to a computer.

Following sample preparation, injection into a reagent vial and measurement in the device the following workflow was followed:



Importantly, for ferrous fumarate, ferrous sulphate, and ferric pyrophosphate, it is recommended to dilute flour sample in 0.2M HCl solution since these iron compounds are only partially soluble in water. NaFeEDTA is soluble in water, hence water can be used as a diluent. It is recommended also to measure intrinsic iron in flour samples using 0.2M HCl. Intrinsic iron is natural iron present in organic samples. In flour the intrinsic iron content may be between 5 and 60 mg/kg.

For sample preparation, the expected concentration in the diluted sample should be in the middle of iCheck Iron linear range (1.5 to 12.0 mg/L). For this it is required to grind the FRK and dilute the powdered sample in a 0.6% NaOH solution followed by incubation for 30 minutes at room temperature. The FRK & NaOH slurry is then further diluted in 0.2M HCl solution shaken for 5 minutes and injected into activated iCheck Iron reagent vial. Detailed protocol can be downloaded here.

RESULTS

Determination of Vitamin A in edible oil samples

A qualitative ring test for Vitamin A in edible oil: 43% of the samples tested positive for Vitamin A, while a notable 57% of the samples lacked detectable levels of Vitamin A.

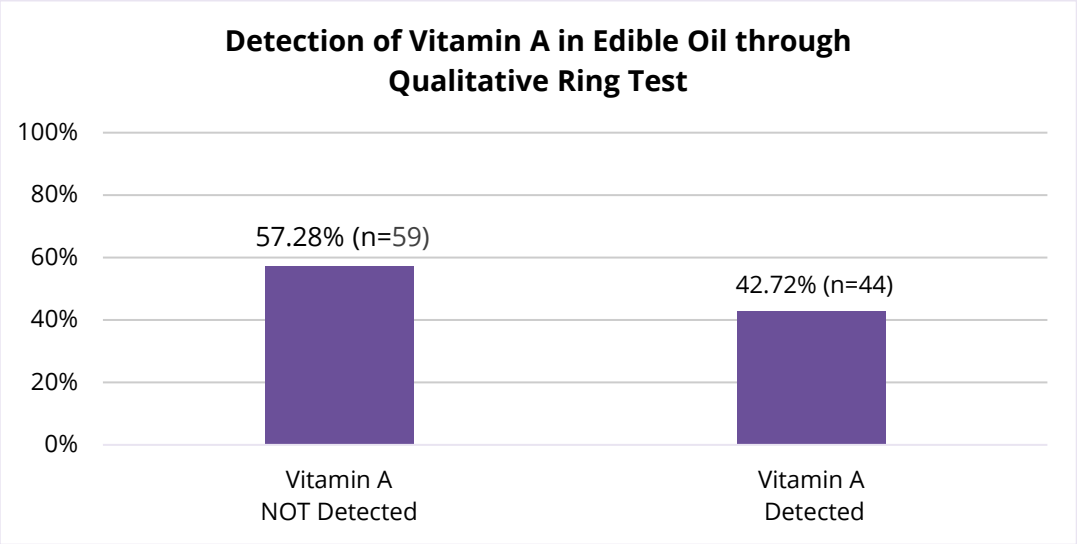


Figure 39: Qualitative assessment of vitamin A in oil with ring test (n=103)

India has a national standard for fortification with Vitamin A in edible oil of 6 – 9.9 µg RE/g (6 - 9.9 mg RE/kg). Considering that iCheck Chroma 3 linear range is 3-30 mg RE/kg, samples containing fortification of vitamin A can be directly tested. Results with iCheck were grouped in the following way:

- Below LOQ: <= 3 µg RE/g
- Fortified below recommended level: 3 - < 6 µg RE/g
- Adequately fortified: >= 6 - <= 9.9 µg RE/g
- Fortified above recommended level: > 9.9 µg RE/g

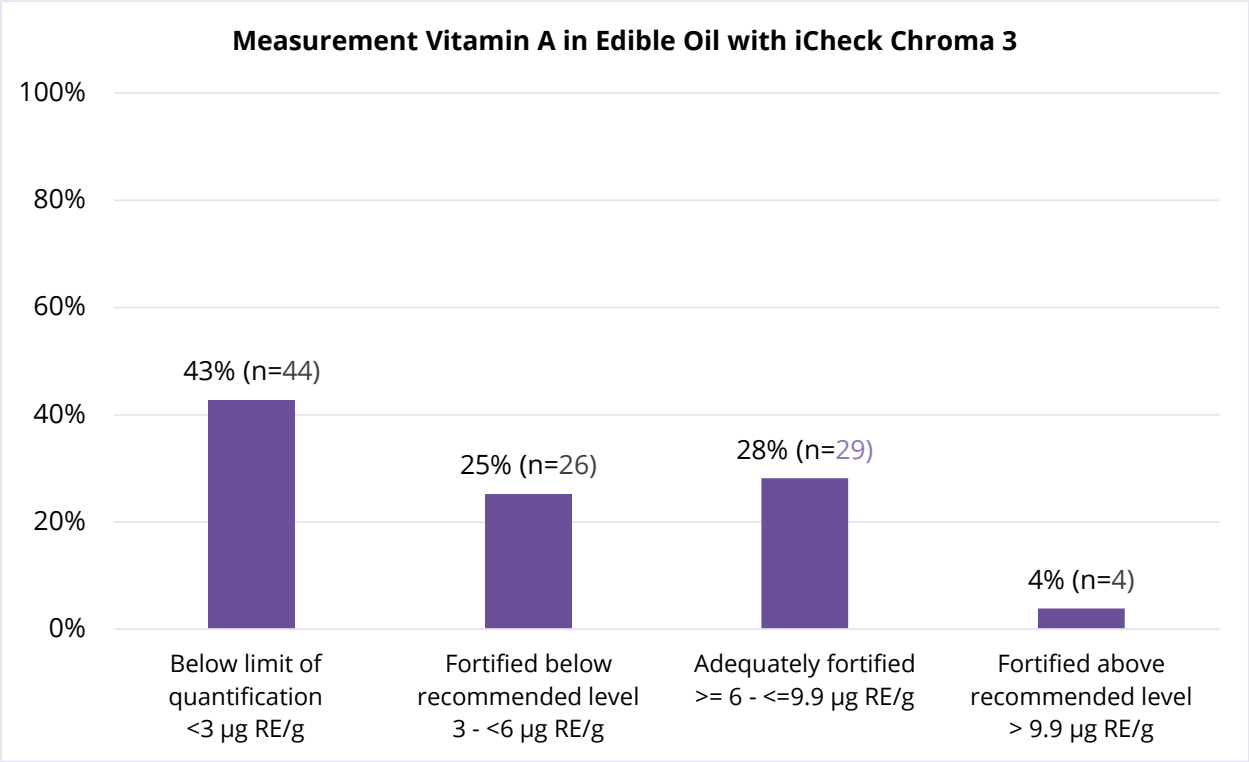


Figure 40: Measurement Vitamin A in Edible Oil with iCheck Chroma 3. (n=103)

The iCheck Chroma 3 results: 43% of the samples have Vitamin A levels below the linear range of 3µg RE/g. 25% contain vitamin A fortification below the recommended national standard between >3.0 and 6 µg RE/g. 28% of all samples tested with iCheck Chroma 3 showed fortification within the national standard range. Fortification above the recommended levels was only present in 4% of cases. The comparison between iCheck Chroma 3 and qualitative ring test results for Vitamin A content in samples show similar results though qualitative results detect lower number of positive for vitamin A samples.

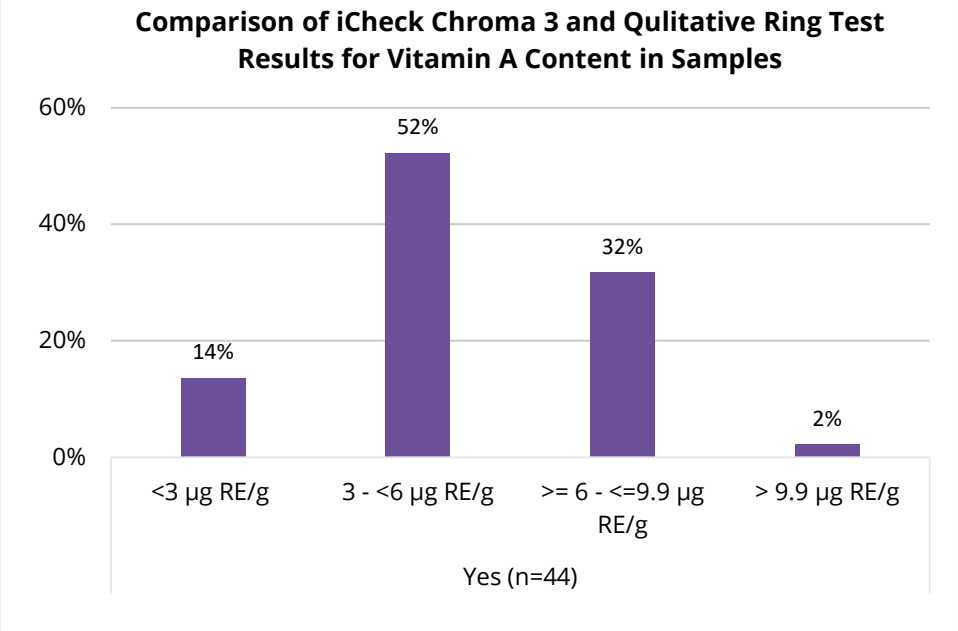


Figure 41: Comparison of iCheck Chroma 3 and Qualitative ring test results for Vitamin A Content in Samples. (Yes, n=44)

These findings indicate that qualitative spot test may miss some fortified samples, especially when the Vitamin A content is low and the visual indicator (blue reaction) is weak, leading to inaccuracies in visual evaluations.

Following the testing of samples with iCheck Chroma 3 and qualitative Vitamin A ring test, 20% of samples were analyzed at local accredited laboratory in India and an external laboratory in Germany for Vitamin A quantification. HPLC was the method used in both local reference laboratories.

Method Comparison – iCheck vs. Local accredited laboratory-India

For comparison purposes, a total of 28 samples tested in all methods: iCheck Chroma 3 and HPLC methods at an external laboratory (Germany) and a local accredited laboratory (India), for vitamin A quantification are compared in the following section.

Firstly, the comparison between iCheck Chroma 3 and HPLC measurements in a local accredited laboratory-India confirm a similar distribution of samples in all categories. Moreover, iCheck Chroma 3 and HPLC quantified samples adequately fortified (>=6 - <=9.9 µg RE/g) in a similar range: 25% (n=7) and 21% (n=6), respectively. In this sense, most of samples in both datasets appear to be fortified below the recommended standard or below the LOQ. iCheck Chroma 3 quantified 39% (n=11) of samples below standard and an important 29% (n=8) of samples below the LOQ. A similar distribution for HPLC samples tested at the local accredited laboratory with 57% (n=16), and 21% (n=6) of samples falling in those categories.

Interestingly, only iCheck Chroma 3 quantified a 7% (n=2) of samples fortified above the national recommended level (>9.9 µg RE/g). Meaning that most of samples keep low levels

of vitamin A, and this supports the idea that qualitative ring test can be challenged to provide reliable data within this range of fortification.

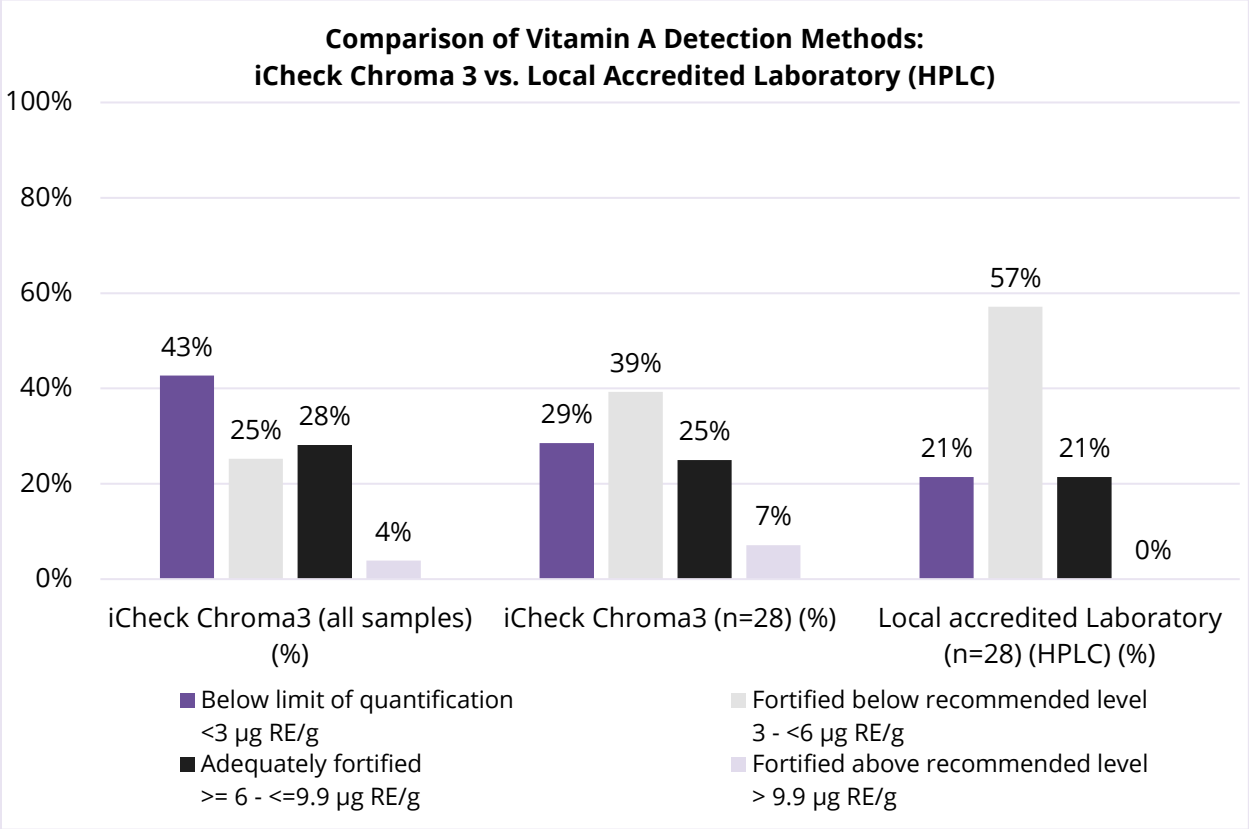


Figure 42: Comparison of Vitamin A Detection Methods: iCheck Chroma 3 (all samples n=104) vs. iCheck Croma 3 (n=28) vs. Local accredited laboratory (n=28)

The comparison of Vitamin A detection methods using samples tested by iCheck Chroma 3, and local accredited laboratory (HPLC) reveals consistent results. The variability in results between iCheck Chroma 3 and the local accredited laboratory HPLC is shown below using a linear correlation.

This correlation between iCheck Chroma 3 and the local accredited laboratory in India using HPLC is poor, with a Pearson correlation coefficient of -0.41, and a R^2 value of 0.0477 suggesting a low level of correlation.

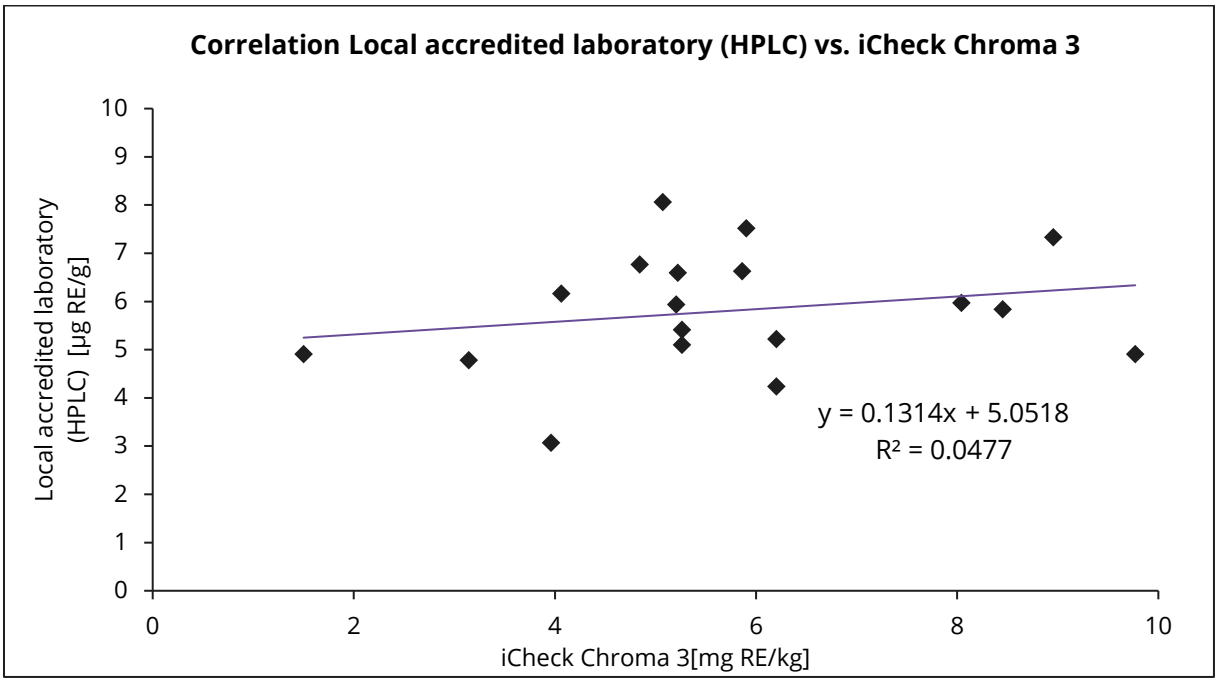


Figure 43: Comparison of the iCheck Chroma3 vs. Local accredited laboratory (HPLC) reference method.

This method comparison underscores the need for standardized testing protocols and cross-validation of methods to ensure accurate and reliable assessment of Vitamin A fortification in edible oils.

Method Comparison – iCheck vs. External laboratory-Germany

The External laboratory-Germany HPLC method reported that 46% (n=13) of the samples were fortified below the recommended level, and 46% were below the quantification limit, with no samples meeting the adequate fortification level. Only 7% (n=2) appear fortified within the national standard.

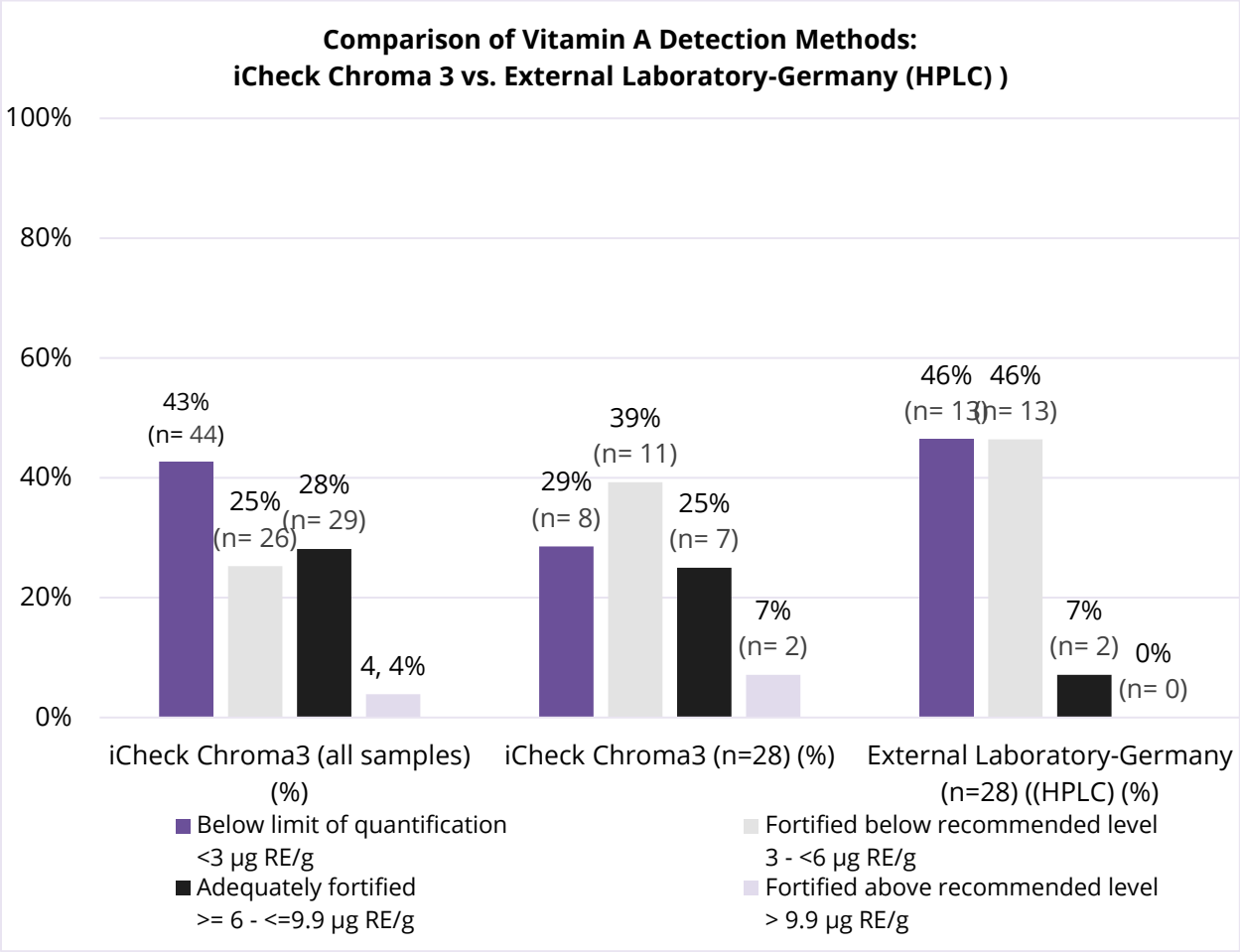


Figure 44: Comparison of Vitamin A Detection Methods: iCheck Chroma 3 (all samples n=104) vs. iCheck Croma 3 (n=28) vs. External laboratory-Germany (n=28) vs. Local accredited laboratory (n=28)

The following Bland-Altman plot compares the Vitamin A measurements between the external laboratory HPLC method and iCheck Chroma 3.

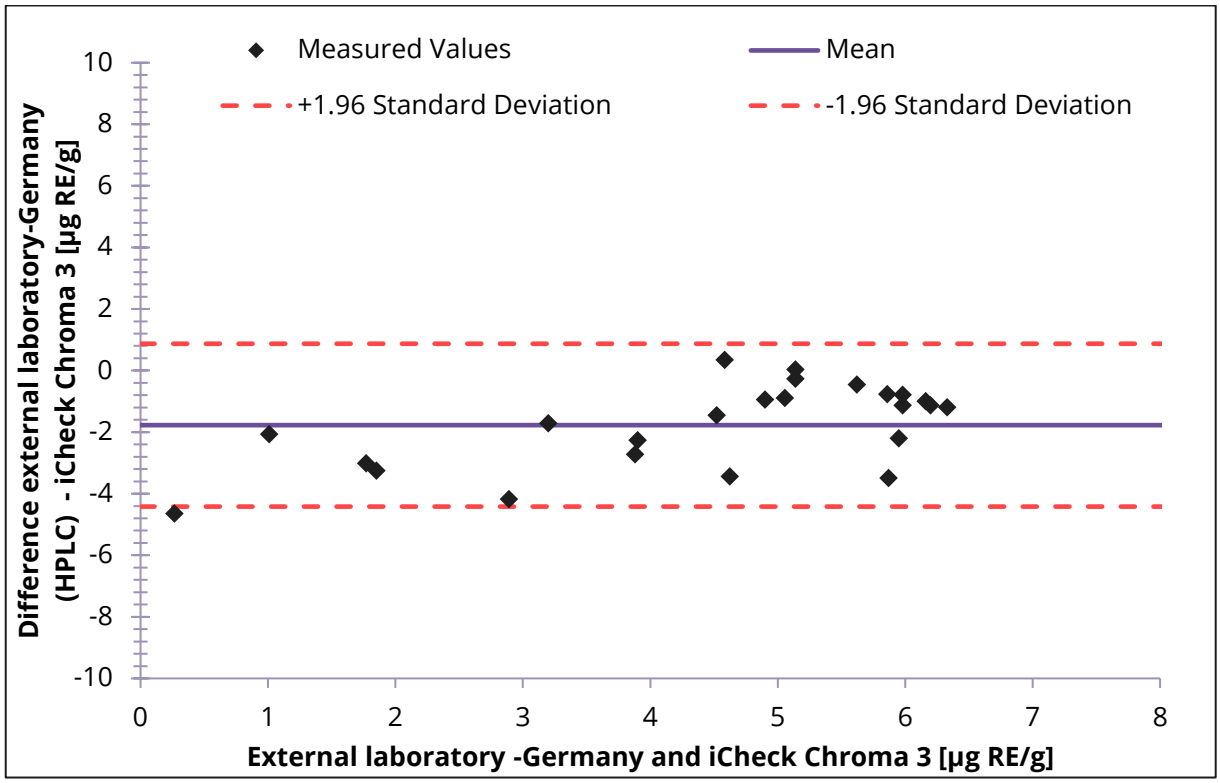


Figure 45: Comparison of the iCheck Chroma3 vs. External laboratory-Germany (HPLC) reference method.

The average difference between the two methods is -1.77 µg RE/g, suggesting that iCheck Chroma 3 tends to slightly overestimate compared to the External laboratory-Germany HPLC method. With a standard deviation of 1.32 µg RE/g and limits of agreement ranging roughly from -4.41 to 0.87 µg RE/g, the variability between the two methods remains within an acceptable range for the majority of samples.

The correlation plot further supports the degree of agreement, with a Pearson correlation coefficient of 0.66, indicating a moderate correlation between the iCheck Chroma 3 and External laboratory-Germany HPLC results. The R^2 value of 0.432 suggests a moderate level of correlation, implying that despite some variability, the iCheck Chroma 3 provides results that are reasonably consistent with the HPLC method.

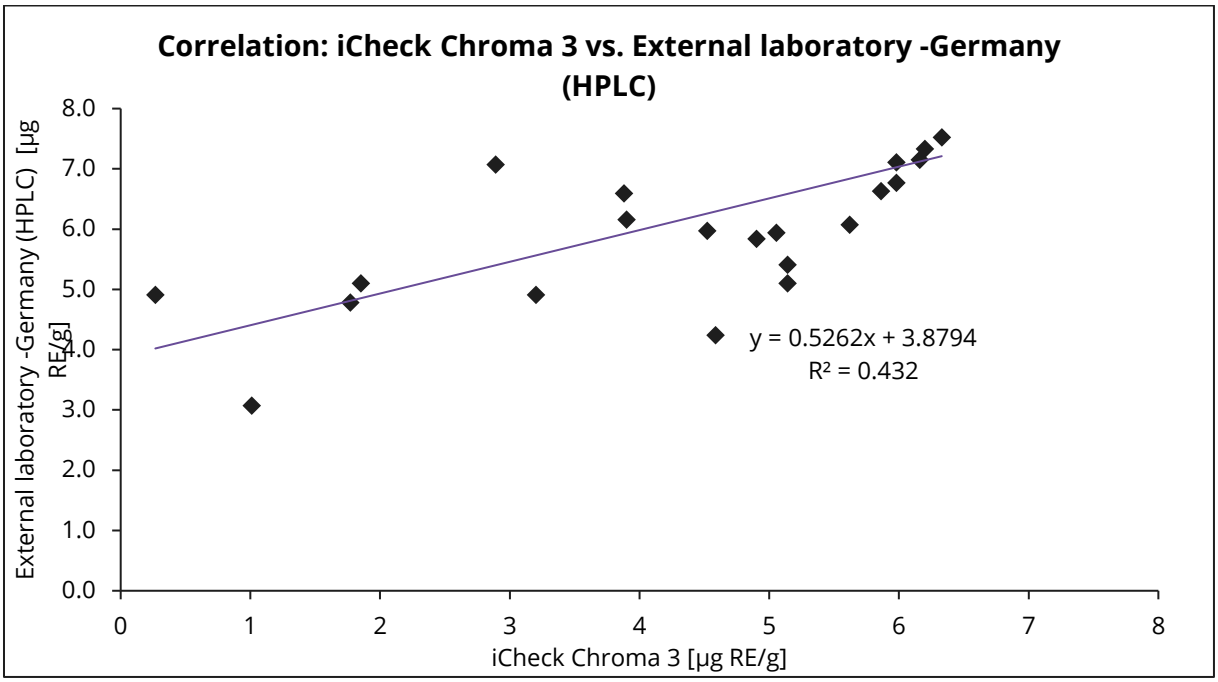


Figure 46: Linear correlation between the Vitamin A measurements from the iCheck Chroma 3 device and the External laboratory-Germany HPLC method.

The correlation between External laboratory Germany and the local accredited laboratory in India HPLC is also poor. Both methods require protocol optimization for measurement of vitamin A in edible oils.

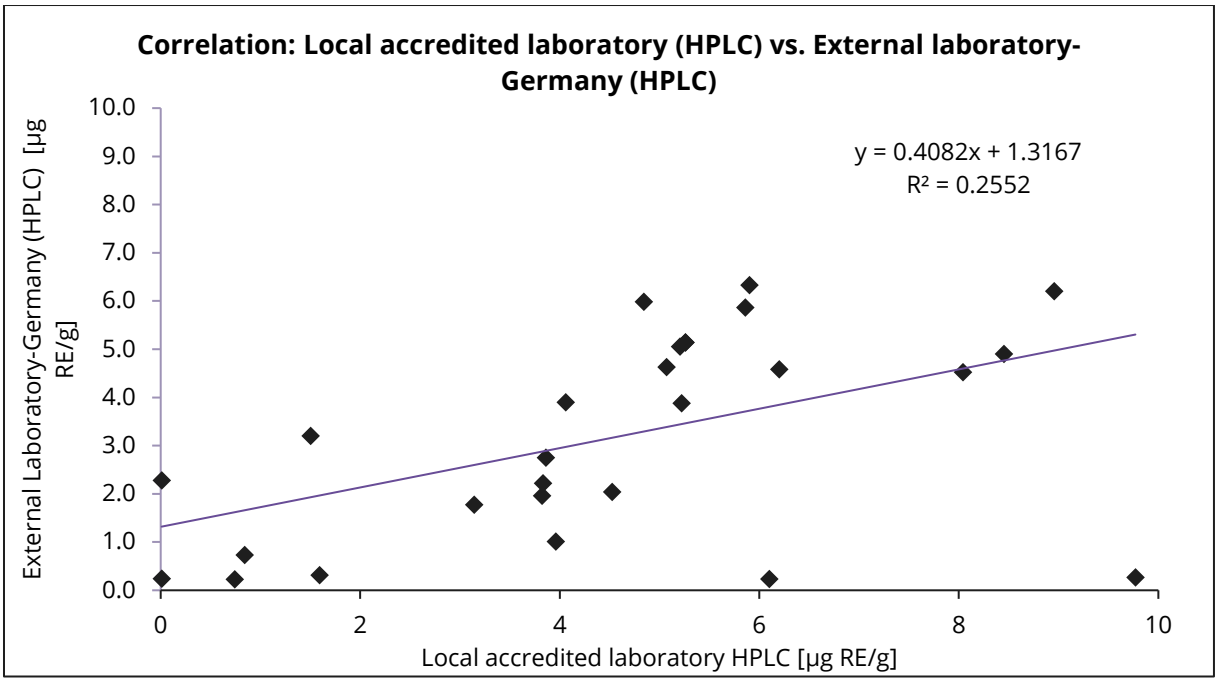


Figure 47: Comparison of Local accredited laboratory (HPLC) vs. External laboratory Germany (HPLC) reference method.

iCheck Chroma 3 and results at the local accredited and external laboratories recovery and precision were assessed using spiked control samples. External laboratory in Germany as observed in earlier internal studies tends to have lower recoveries, while local accredited laboratory shows inconsistent recovery. Further tests should be performed.

Target concentration (added vitamin A at BioAnalyt lab)	iCheck Chroma 3	HPLC at External laboratory, Germany	HPLC at Local accredited laboratory, India
7.5 µg RE/g	7.9±0.4 µg RE/g (105% recovery) by BioAnalyt	6.5±0.03 µg RE/g (87% recovery)	8.33 µg RE/g (111% recovery)
10 µg RE/g	10.9±0.04 µg RE/g (109% recovery) by BioAnalyt	Not sent	5.05 µg RE/g (50.5% recovery)
	10.2±0.4 µg RE/g (102 recovery) by local analysts		Not sent

Fresh samples directly from producers

GAIN additionally collected fresh samples from local producers of fortified oils of different types within days of testing (n=14). The samples were tested with iCheck Chroma 3 at AVON labs and sent to external laboratory in Germany. The correlation is higher between those results, suggesting that oil quality impacts result significantly.

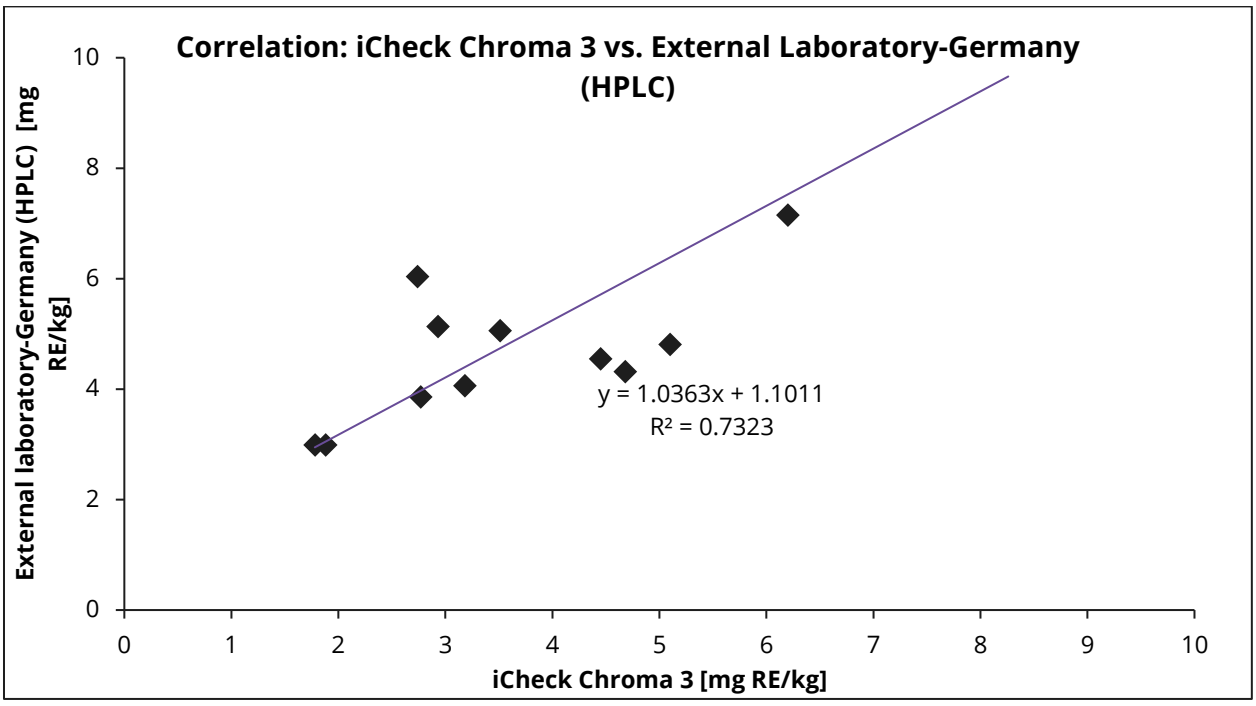


Figure 48: Comparison of iCheck Chroma 3 vs. External laboratory Germany (HPLC) reference method in newly collected edible oil samples from local producers in India, provided by GAIN during the testing phase of the project (n=14).

Determination of Iron in Fortified Rice Kernels

A qualitative spot test for Iron in FRK samples revealed a significant level of fortifications across the samples, with only 8% of the samples tested negative for Iron.

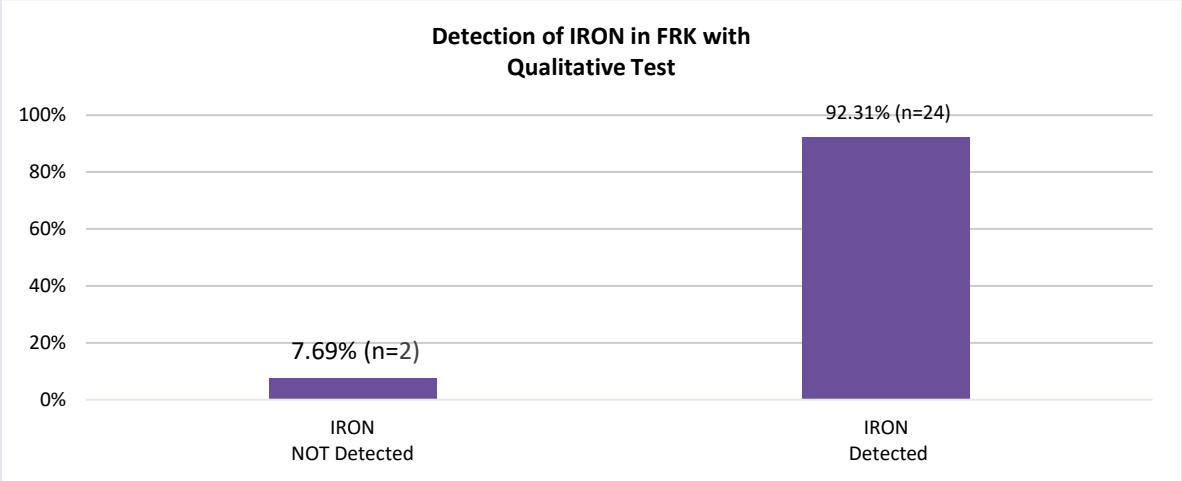


Figure 49: Qualitative assessment of iron in FRK with colorimetric spot test (n=26)

India has a national standard for fortification with Iron (micronized ferric pyrophosphate) in FRK of 2800 - 4250 mg/kg. Considering that iCheck Iron linear range 1.5-12.0 mg/L and the dilution factor of 525, results with iCheck were grouped in the following way:

- Below linear range <=787.5 mg/kg*
- Below national standard >787.5 - <2800 mg/kg
- According to national standard 2800 - <= 4250 mg/kg
- Above national standard >= > 4250 mg/kg

* LOQ iCheck Iron is 1.5 mg/L at a dilution factor of 525 used for all samples = 787.5mg/kg.

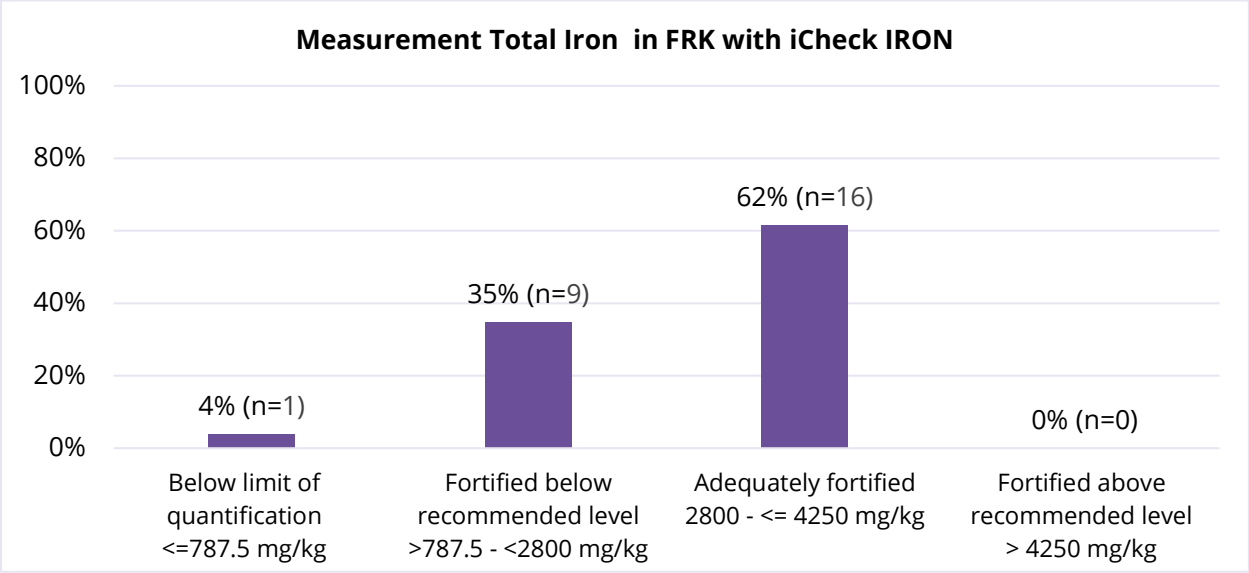


Figure 50: Measurement Iron in FRK with iCheck Iron. (n=26)

The measurement of total iron in fortified rice kernels (FRK) using the iCheck IRON: all but one sample had iron concentration above 787mg/kg. The majority, 62%, were within the national standard. Given the small samples size that was received from PATH (n=26 samples), instead of 20%, all samples were sent for testing with other methods at accredited laboratories.

Method Comparison – iCheck vs. Local accredited laboratory-India

When comparing iCheck Iron with local accredited laboratory in India, a similar distribution is identified. Most samples appear to contain high levels of Iron, with 62% (n=16) of samples within national standard and 35% (n=9) with levels below the standard. Similarly, results at a local accredited laboratory-India measured 50% (n=13) of samples as within standard, 46% (n=12) below standard.

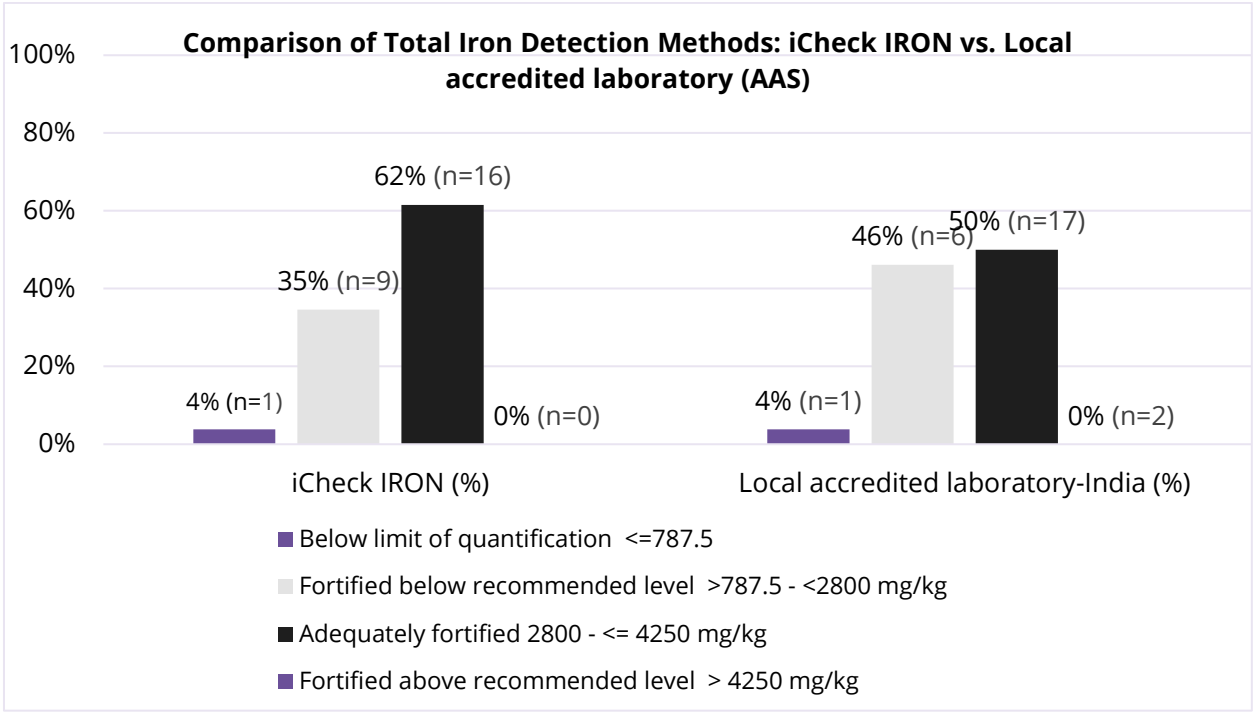


Figure 51: Comparison of iron quantitation methods: iCheck Iron (n=26) vs. Local accredited laboratory-India (AAS) (n=26).

The correlation plot between iCheck Iron and local accredited laboratory AAS method has a Pearson correlation coefficient of 0.76, indicating a moderate positive correlation between the two methods.

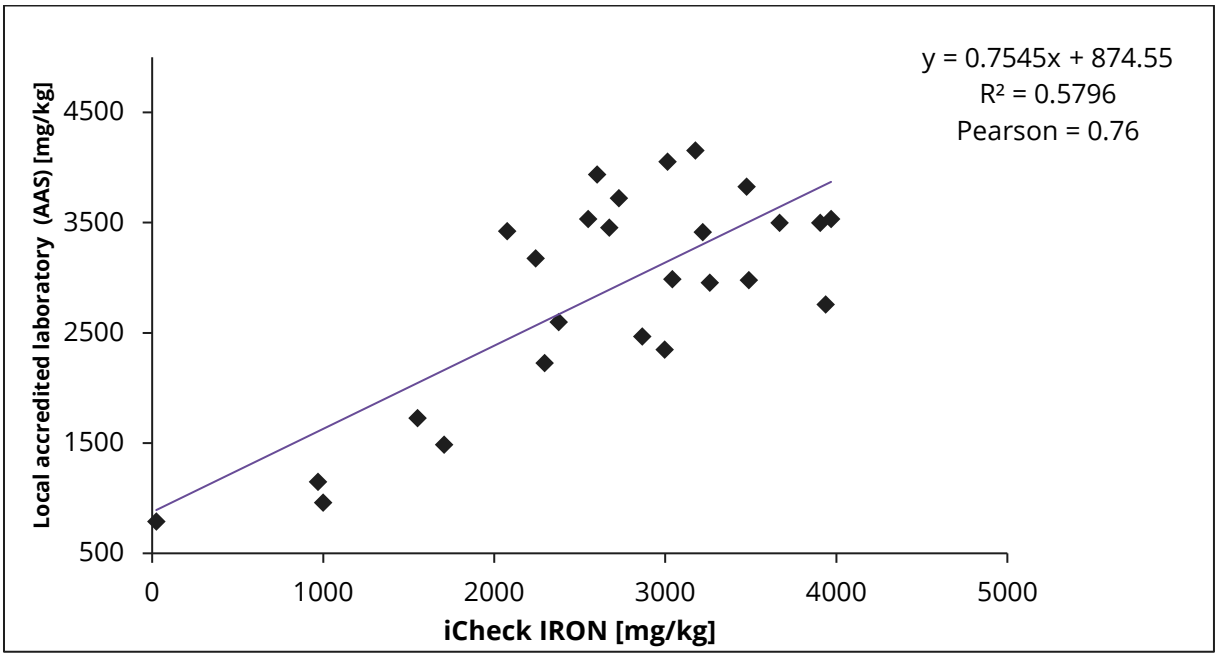


Figure 52: Linear correlation between the Iron measurements from iCheck Iron vs. Local accredited laboratory (AAS) reference method.

Method Comparison – iCheck vs. External laboratory-Germany

The External laboratory-Germany ICP method reported that 65% (n=17) of the FRK samples were within national standard, only 23% (n=6) were below the standard. Overall, those results are consistent with the distribution of samples quantified at local accredited laboratory-India.

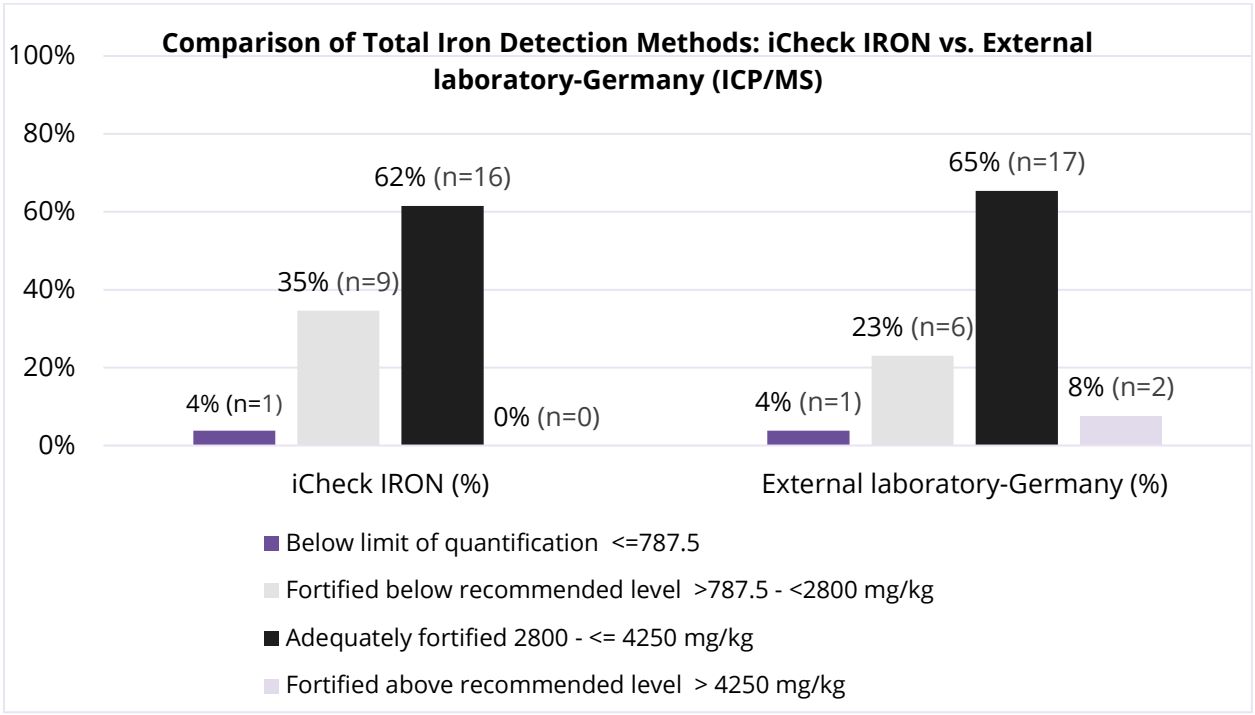


Figure 53: Comparison of iron quantitation methods: iCheck Iron (n=26) vs. External laboratory-Germany (ICP/MS) (n=26).

Moreover, the correlation between the External laboratory-Germany ICP/MS method and iCheck Iron is stronger with Pearson correlation coefficient of 0.80, and a R^2 value of 0.644 suggesting a moderate level of correlation,

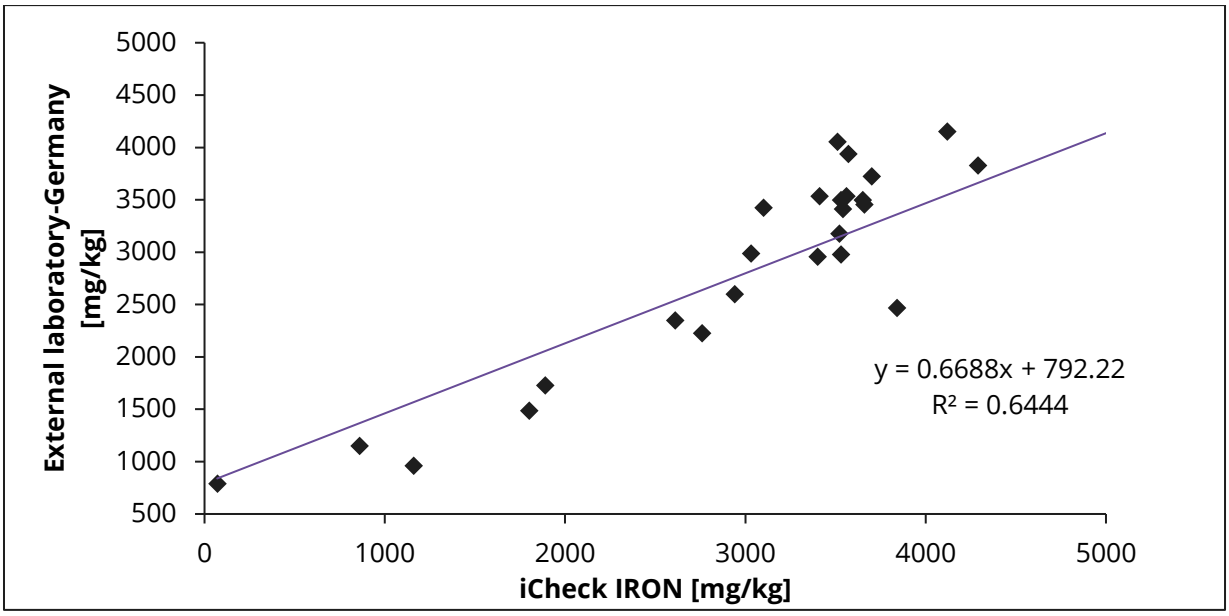


Figure 54: Linear correlation between the Iron measurements from iCheck Iron vs. External laboratory -Germany (ICP/MS)

The correlation between the local accredited laboratory and External laboratory-Germany has slightly lower Pearson coefficient of 0.76.

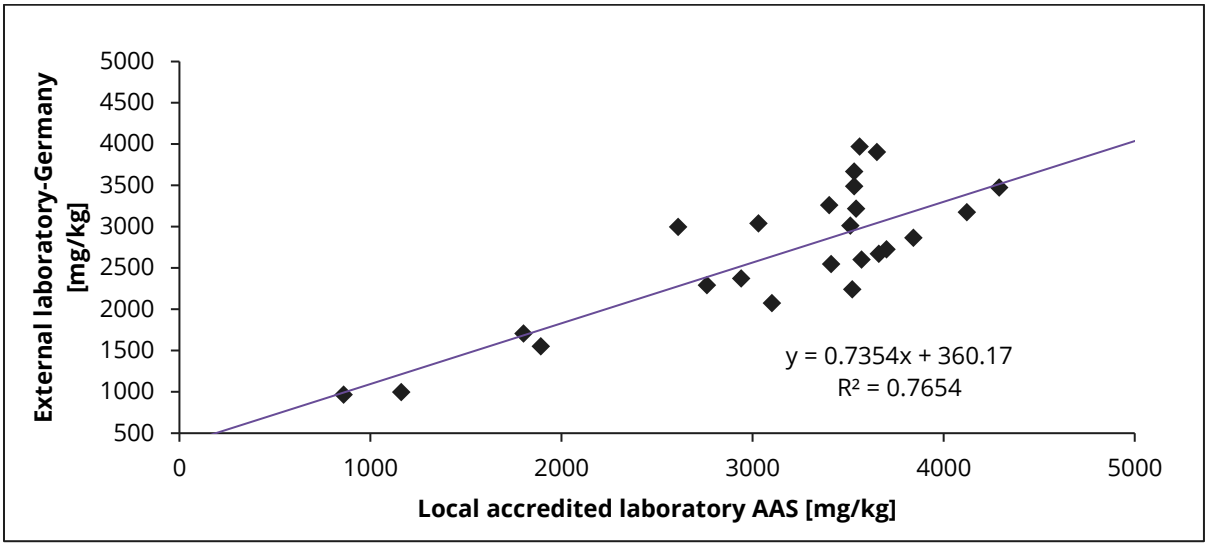


Figure 55: Linear correlation between the Iron measurements from External laboratory -Germany (ICP/MS) vs. Local accredited laboratory (AAS) reference method.

The assessment of recovery could not be performed at the local laboratory with AAS as no spiked samples were tested.

Table 4: Iron recovery (%) in FRK samples using iCheck Iron and a External laboratory-Germany ICP/MS method.

Target concentration (Fe in FRK), assessed by External /BioAnalyt	iCheck Iron	ICP MS at External laboratory, Germany	AAS at Local accredited laboratory, India
3000 mg/kg	2713±141 mg/kg (90% recovery) by trained Analysts	3090±28 mg/kg (103% recovery)	Unknown
3000 mg/kg	2996±159 mg/kg (100% recovery) by BioAnalyt analysts		

Local labs analysis with AAS of iron in FRK performed better than HPLC analysis of vitamin A in oil. Although here too it is recommended to further refine the protocol to improve recovery. Blinded duplicates sent to ICP Germany (2 samples) have CV of 4% while blinded duplicates (6 samples) analyzed by AAS locally had CV of 31%. The duplicates (3 samples) when reported by local lab with AAS has CV of 0%.

Within the preparation of this project, during market sizing, data from FRK manufacturers and FRK suppliers in India were collected (obtained at: https://foscos.fssai.gov.in/frk-manufactures, and http://annavitran.nic.in/FR/frkSupplierRpt), and its analysis enabled us to narrow down regions of interest where a potential fortification assessments could be used in a near future based on the local production capacity and/or product availability.

Table 5: Summary of FRK suppliers. Commonly, a supplier purchases FRK from a manufacturer to blend it with regular rice at a 1:100 or 1:200 ratio for distribution and commercialization.

State Name	# FRK Suppliers	% FRK Suppliers in India	TOTAL Production Capacity per State	% TOTAL Production Capacity in India	AVG Production Capacity per State	# FRK Suppliers ABOVE AVG Production Capacity
PUNJAB	131	36	2256	**40**	17	33
HARYANA	45	12	672	**12**	15	21
UTTAR PRADESH	39	11	573	**10**	15	11
WEST BENGAL	18	5	431	**8**	24	4
TELANGANA	11	3	356	**6**	32	2
CHHATTISGARH	32	9	260	**5**	8	10
Total	276	76	4548	**80**		81

A sampling from a combination of states, could enable to have a broader country perspective for mapping and surveillance of FRK production, as shown in the following chart:

Table 6: Mapping of market coverage of FRK Manufacturer in India.

State Name	# FRK Manufacturers	% FRK Manufacturers in India	% MARKET COVERAGE - RFK MANUFACTURERS				
Punjab	323	29%	**43%**	**54%**	**65%**	**71%**	**81%**
Chhattisgarh	150	14%					
Tamil Nadu	124	11%					
Uttar Pradesh	116	11%					
Haryana	67	6%					
Bihar	60	5%					
Madhya Pradesh	49	4%					

Assessment of fortification levels by the brands and geography was not done as the data was not available. Altogether, analysis of the reduced amount of FRK samples enabled a narrow insight into FRK iron levels in India

INDIA: LIMITATIONS AND CHALLENGES DURING PLANNING AND IMPLEMENTATION

Challenges	Impact on	Control Measures & Mitigation	Recommendations
Delay in project implementation due to the requirement of using FSSAI approved methods for rapid assessment.	Timeline	FSSAI requested QuImpact to complete the RAFT process. IIT Delhi / NIFTEM was suggested based on request from QuImpact for expediting the process	Initial alignment of local partners about the goals of rapid assessment. Accreditation of the iCheck methods by FSSAI or AOAC.
Samples could only be sourced from already ongoing studies by GAIN and PATH/NI due to lack of specifically allocated budget and difficulty aligning the timelines.	How representative were the samples of the market; Stability of vitamin A	It was recommended to freeze the oil samples until the rapid assessment by BioAnalyt. It cannot be confirmed if this was done.	Collection must be planned and aligned with the testing period for time sensitive samples such as fortified oil.
Local elections in India (April-June) may pose restrictions on conducting this type of analysis in the regions. Aligning the timelines of all the involved parties made it difficult to get dates that did	Timeline	QuImpact negotiated with Avon Labs to perform analysis during national holidays.	Timeline planning tat includes national holidays.

not overlap with national holidays.			
Representative sampling of FRK for market assessment was not possible.	How representative were the samples of the market	Only samples from FRK manufacturers that were willing to cooperate with NI/PATH could be collected.	Assistance from local stakeholders to collect the FRK during the active production season and their storage until analysis is possible.

ANNEXES

ANALYSTS TRAINING:

Indonesia

The figure below represents the results of a rapid assessment training session held in Indonesia, focusing on measuring Vitamin A levels in oil using the iCheck Chroma 3 device. Each black dot represents the different sample measurements results obtained by each trained KFI Analysis, including the variability for each analyst. Measurements of concentration of Vitamin A (mg RE/kg) are presented against the expected concentration of Vitamin A from the target sample at 20 mg RE/kg = 66.66 IU/g.

Blue continuous lines show ±13% coefficient of variation, the maximum observed in internal validation of iCheck Chroma 3. Red dashed lines indicate the measurement uncertainty at 95% confidence (+/-30%):

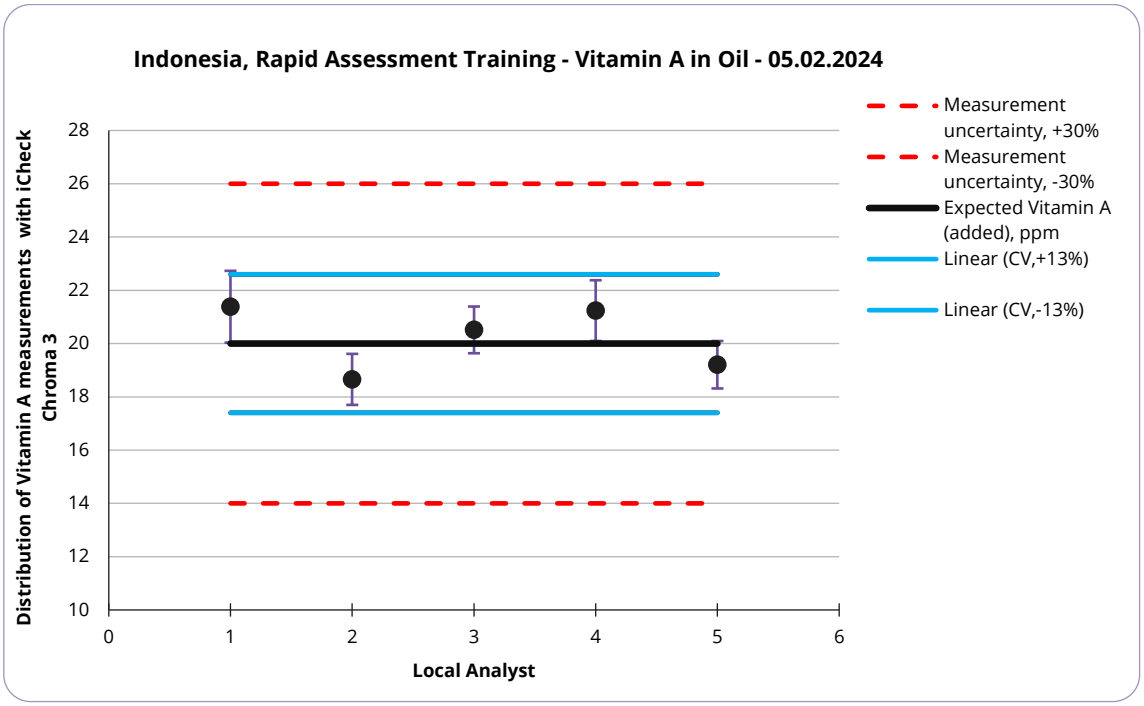


Figure A1: Indonesia, Rapid Assessment Training - Vitamin A in Oil testing with iCheck Chroma 3 - 05.02.2024

The graph shows that most of the Vitamin A measurements were within the acceptable range of variability (±13% CV) around the expected value. The measurements demonstrated that the training was successful in enabling analysts to produce accurate measurements with iCheck Chroma 3.

Kenya

The following figures illustrate the results of a training session held at AMS, focusing on measuring Vitamin A and Iron levels in wheat and maize flour using the iCheck Fluoro and iCheck Iron devices. Each black dot represents the different sample measurement results obtained by each trained AMS analyst, including the variability for each analyst. Measurements for Vitamin A in flour have a coefficient of Variance (CV) of 15%, and a measurement uncertainty (MU) of 30%. The expected concentration was 1.4 mg/kg. Considering a matrix effect of 0.25mg/kg (measured internally) the graph shows the results from AMS analysts as follows: Analyst A: 1.3 mg/kg ±0.03mg/kg, and Analyst B:1.2 mg/kg ±0.03mg/kg:

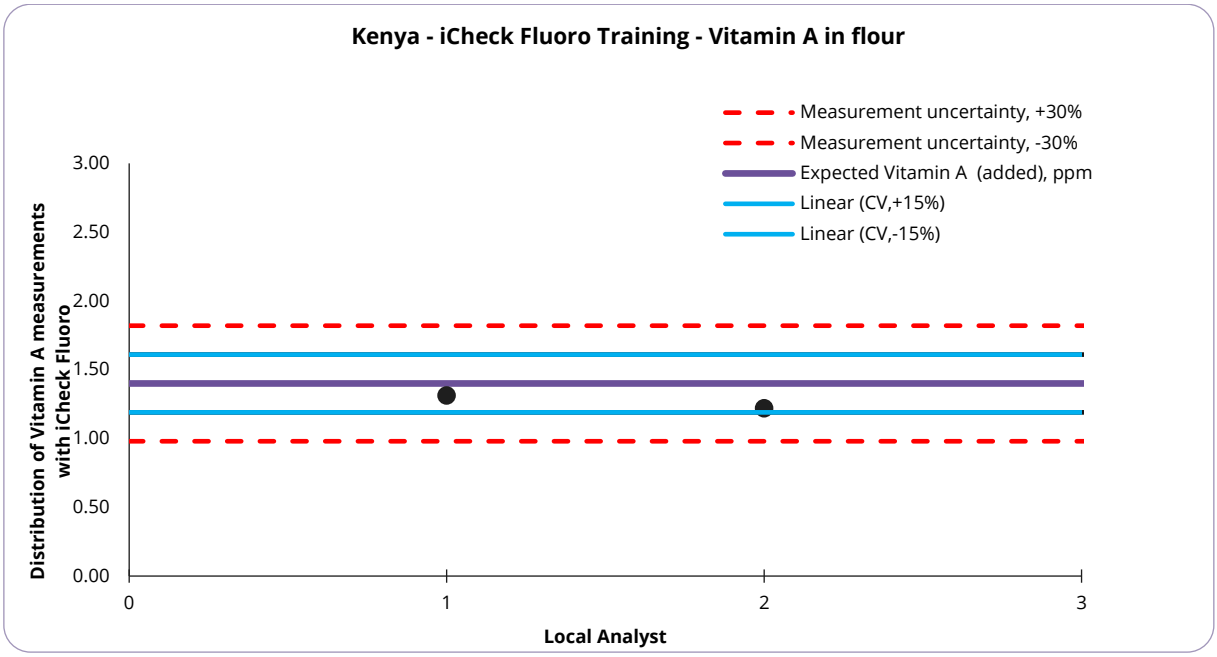


Figure A2: Kenya, iCheck Fluoro Training - Vitamin A in spiked Wheat Flour samples

Training in iron measurements was performed with iCheck Iron with ferrous fumarate spiked flour samples with an added concentration of 60 mg/kg, the intrinsic iron is <10mg/kg. The target coefficient of variation observed in internal validation of iCheck Iron with ferrous fumarate fortified flour (diluted in 0.2M HCl) is +/-9%. Measurement uncertainty at 95% confidence is +/-18%. Results from this training showed the Analyst A averaging 77 mg/kg ±4.5mg/kg, and Analyst B averaging 71 mg/kg ±8mg/kg:

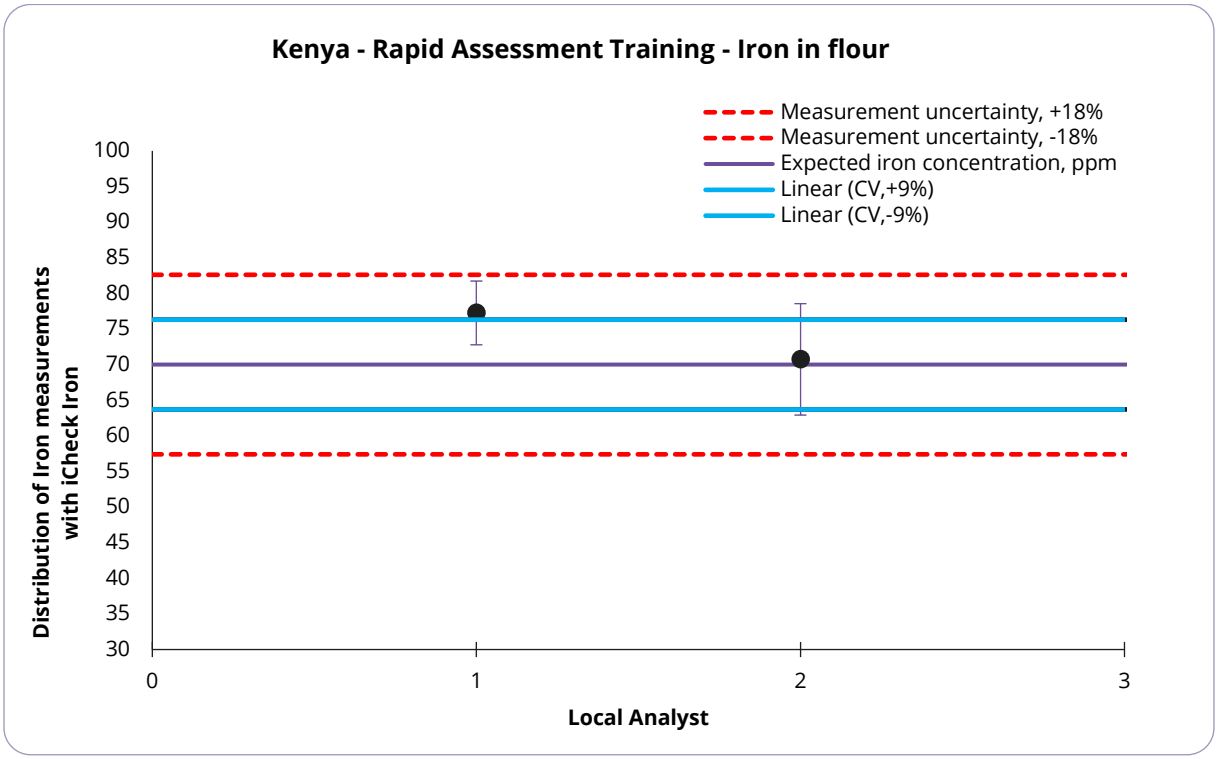


Figure A3: Kenya, iCheck Iron Training – Iron spiked Wheat Flour samples.

The measurements suggested that the training was effective although close attention must be paid to protocols during sample dilution and reaction/extraction of analytes in reagents vials. Rigorous shaking is key, and making sure centrifugation step is effective at removing any particles from the upper phase.

India

The results of a rapid assessment training session held in India are shown in the figure below. The training focused on measuring Vitamin A levels in oil using the iCheck Chroma 3 device and iron in fortified reconstituted kernel using iCheck Iron. Each black dot represents the different sample measurements results obtained by each trained Analyst, including the variability for each analyst.

Measurements of concentration of Vitamin A (mg RE/kg) are presented against the expected concentration of Vitamin A from the target of 10 mg RE/kg. Blue continuous lines show ±13% coefficient of variation, the maximum observed in internal validation of iCheck Chroma 3. Red dashed lines indicate the measurement uncertainty at 95% confidence (+/-30%):

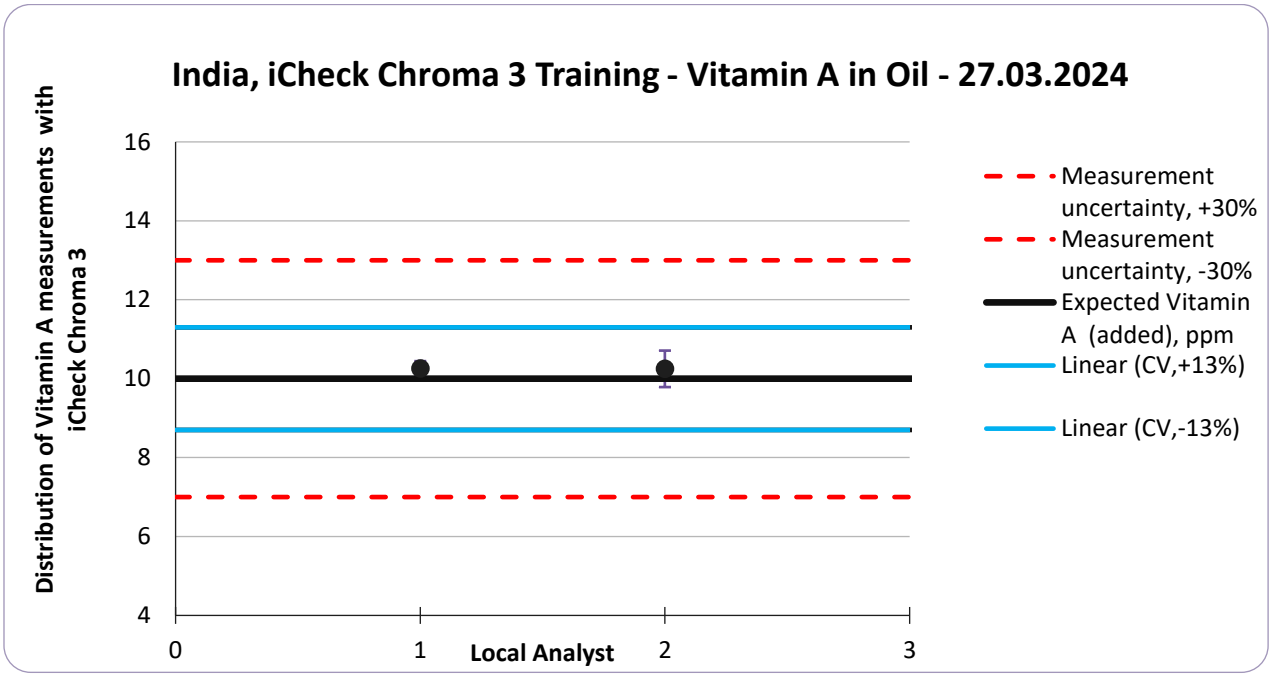


Figure A4: India, iCheck Chroma 3 Training - Vitamin A in Oil - 27.03.2024.

Measurements of concentration of Iron (mg/kg) are presented against the expected concentration of Iron from the target at 3000 mg/kg. Blue continuous lines show ±6% coefficient of variation, the maximum observed in internal validation of iCheck Iron with FRK. Red dashed lines indicate the measurement uncertainty at 95% confidence (+/-14%).

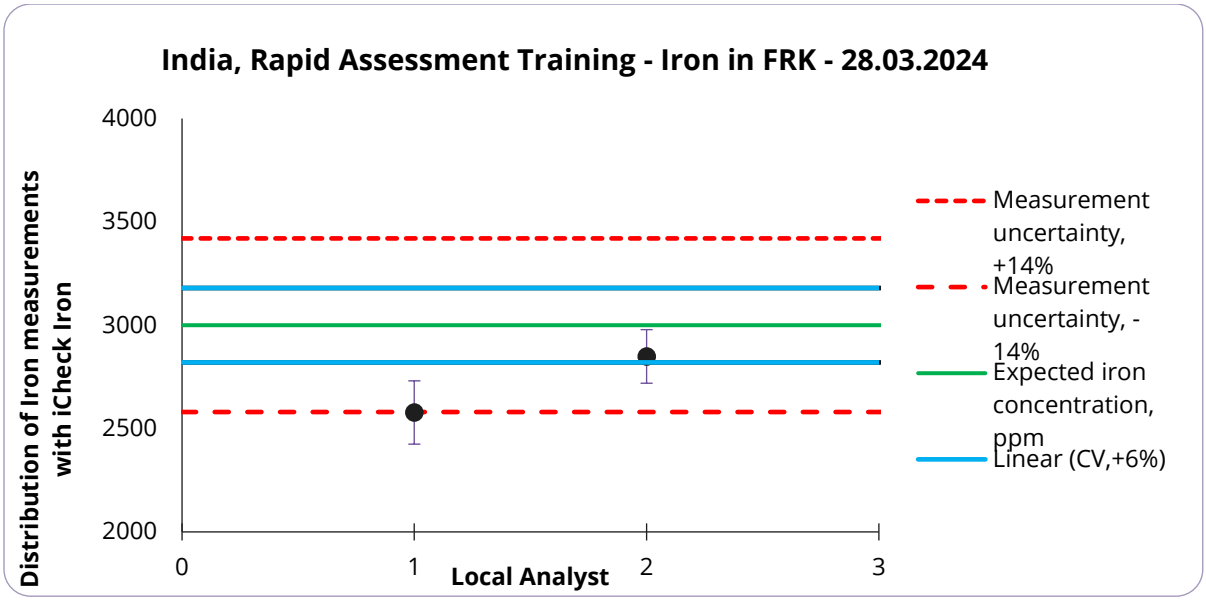


Figure A5. India, iCheck Iron Training – iron in FRK - 27.03.2024.

The CV for both methods and both analysts is within acceptable range. The recovery of iron in FRK is in the lower range, however still within MU. More careful execution of sample preparation protocol for FRK was recommended.

SAMPLES COLLECTION PROTOCOLS

Indonesia

Jakarta: 63% (Sumatra, Banten, Java/except East Java)
Surabaya: 37% (East Java, Kalimantan, Sulawesi Bali, NTB, NTT, Maluku and Papua)

Sample collection protocol						**Sample to be validated at local reference laboratory**	
Brand	Producers	Market share	Samples size	Jakarta	Surabaya	Jakarta	Surabaya
Brand		85%	425	268	157	53	32
Minyak Kita		15%	75	47	28	10	5
TOTAL			**500**	**315**	**185**	**63**	**37**
I. **Branded Oil**							
(i) Bimoli	Salim Group	21%	105	66	39	13	8
(ii) Tropical	BKP	15%	75	47	28	9	5
(iii) Filma	Sinarmas	14%	70	44	26	9	5
(iv) Sania	Wilmar	11%	55	35	20	7	4
(v) Rose Brand	TBL	8%	40	25	15	5	3
(vi) Sunco	Musim Mas	6%	30	19	11	4	2
(vii) Kunci Mas	Sinarmas	4%	20	13	7	2	2
(viii) Camar	RGE	3%	15	9	6	2	1
(ix) Goldie	Wilmar	2%	10	6	4	1	1
(x) Palma	Darmex Agro	1%	5	3	2	1	1
II. **Minyak Kita**		15%	75	47	28	10	5
TOTAL			**500**	**315**	**185**	**63**	**37**

(i) Edible oil sample collection criteria

- Brands must include a fortification mark
- Collect a maximum of **5 different lots per brand, including 3 samples from one lot** of the brand,
- Collect unbroken packages of each brand with a **minimal weight or volume or 500 g or 500 ml respectively**. Include one unbranded sample (Registered Minyak-kita)
- Prioritize the collection based on market share representation and relevance in the market
- Collect samples from Jakarta and Surabaya region
- Ensure the documentation and registration of collection data

Samples of branded palm cooking oil should be collected from retails, such as,

- Large-scale retail: supermarket, hypermarket, department store
- Small-scale retail: traditional market, or kiosk / warong

- Modern retail: indomaret, alfamart

If possible include the following brands

- Ensure that the following brands are collected
  Bimoli, Tropical, Filma, Sania, Sunco; Rose Brand, Kunci Mas, Camar, Goldie, Palma (as per the above table)
- If possible select Minyak kita samples based on possible differences of producer. Those Minyak kita sample of one producer should be treated like a branded sample regarding to lot and number of samples taken per producer
- In addition to the branded oil samples, please collect samples from loose, unbranded oil

FIGURES

TABLES

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