

FIELD EXPERIENCE FROM DESIGNING AND IMPLEMENTING A COLD-CHAIN SYSTEM FOR THE 2021 NIGERIA NATIONAL FOOD CONSUMPTION AND MICRONUTRIENT SURVEY

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ABSTRACT

Improving nutrition is a key component of efforts to promote sustainable development and improve health and well-being. However, it is impossible to effectively improve nutrition in a population without monitoring the nutrition status of vulnerable groups. Monitoring approaches such as micronutrient surveys are critical to allow stakeholders determine the prevalence of malnutrition and identify micronutrient deficiencies of public health importance and at-risk groups. In this regard, the 2021 Nigeria National Food Consumption and Micronutrient Survey (NFCMS 2021) collected biological samples from children aged 6-59 months, adolescent girls, as well as pregnant and non-pregnant women of reproductive age. The micronutrients of interest were retinol, thiamine, riboflavin, cobalamin, folate, iodine, iron, and zinc. Quantitative 24-hour dietary recall data were also collected from children aged 6-59 months, pregnant and non-pregnant women of reproductive age. To achieve its goal, the NFCMS 2021 had to overcome some hurdles. Therefore, the current study details the challenges of developing a cold-chain system for the survey and novel strategies employed to address them. The main challenges encountered included collecting, transporting, processing, storing, and analyzing over 51,143 samples from enumeration areas with unreliable electricity, difficult terrain, and far from functional health facilities. The survey successfully designed and implemented a cold-chain system across 364 enumeration areas in all 36 states and the Federal Capital Territory. This can be attributed to several factors. First, the team reviewed and documented the survey's cold-chain requirements. Second, stakeholder engagement with the federal and state governments allowed the use of Ministry of Health cold stores. This was followed by proximity mapping of survey enumeration areas to all existing cold stores, a visit to verify their suitability, and the selection of the best cold stores for the survey. The survey also procured the right equipments, and trained teams on cold-chain logistics, best practices, troubleshooting, and communication. An innovation of the survey was the use of checklists for decision-making and real-time temperature data loggers and dashboards for monitoring the cold-chain. Using detailed contingency plans for cold-chain logistics and leveraging cost-effective technologies can improve cold-chain transparency, efficiency, and integrity in resource-constrained settings, as was the case of the NFCMS 2021. The survey identified an unmet demand for a detailed manual with updated guidance on designing a cold-chain system for a nutrition survey.

Key words: Cold-chain system, micronutrient survey, resource-constrained settings, Nigeria, Africa, NFCMS 2021



INTRODUCTION

Malnutrition, including both undernutrition and overnutrition, is a major contributor to the global burden of disease and a leading cause of death and disability [1]. In Africa, nutrition is a critical development issue because hunger on the continent has a significant economic cost. In addition, poor nutrition can have severe consequences for individuals and communities, such as increased morbidity, mortality, and impaired cognitive and physical development, resulting in reduced productivity and economic development [2]. Improving nutrition is, therefore, a key component of efforts to promote sustainable development and improve health and well-being [3].

It is impossible to effectively improve nutrition in a population without monitoring the nutrition status of vulnerable groups, for example, boys and girls aged 6-59 months, adolescent girls, women of reproductive age, and pregnant women. Using monitoring approaches such as micronutrient surveys allows stakeholders to determine the prevalence of malnutrition, and identify micronutrient deficiencies of public health importance and at-risk groups. This information is critical for informing the development of agriculture, food, and nutrition policies and programs to address malnutrition. Additionally, micronutrient surveys allow stakeholders to track trends in malnutrition over time, identify potential barriers to adequate micronutrient intake, and evaluate the effectiveness and impact of existing interventions. This evidence is essential for making evidence-based decisions on targeting interventions, allocating resources, and adapting nutrition and public health strategies as needed [4].

Although micronutrient surveys can include anthropometry and dietary assessments, and surveys of health and nutrition indicators, their main focus is the assessment of biomarkers for micronutrients of interest such as iron, iodine, folate, vitamin A, B12, and health-related issues, for example, anemia, iron deficiency, and iron deficiency anemia [4]. Several factors, such as cost, human capacity, and laboratory capacity, can limit the scope and scale of the biomarker component of a micronutrient survey. Chief among these is the ability of survey teams to collect and handle biological samples from the field to the laboratory under appropriate cold-chain conditions. While systems exist in many low and middle-income countries to transport temperature-sensitive materials such as vaccines from central locations to many peripheral sites, often no countrywide systems exist to do the reverse [5]. In addition, in many African countries, including Nigeria, enumeration areas may have unreliable electricity, some may be difficult to access due to difficult terrain, and some may be far from functional health facilities that



could support sample handling and transportation. Therefore, the challenge for a micronutrient survey in these resource-constrained settings is designing and implementing a cold-chain system to maintain sample potency. This methodology paper addresses this challenge by documenting the successful experience of the 2021 Nigeria National Food Consumption and Micronutrient Survey (NFCMS 2021).

The NFCMS 2021 is the most comprehensive cross-sectional population-based national survey in Africa to date. The primary objective of the survey was to assess the micronutrient status, anthropometry, and dietary intake of women of reproductive age (WRA) aged 15-49 years, including pregnant and lactating women and children (aged 6-59 months) as well as the micronutrient status of non-pregnant adolescent girls (aged 10-14 years) and to identify key factors associated with poor nutrition in these populations. In addition to collecting quantitative 24-hour dietary recall data in children aged 6-59 months as well as pregnant and non-pregnant women of reproductive age, blood, urine, and stool samples were collected. The survey measured anemia, glycated hemoglobin, *Helicobacter pylori*, helminths, hemoglobin genotype, malaria, plasma glucose, retinol, thiamine, riboflavin, cobalamin, folate, iodine, iron, inflammation markers [C-reactive protein (CRP) and alpha(1)-Acid glycoprotein (AGP)], and zinc status across four target groups that included adolescent girls [6]. The challenge was collecting, transporting, processing, storing, and analyzing over 51,143 samples from enumeration areas with unreliable electricity, difficult terrain, and far from functional health facilities. Following a successful design and implementation of a cold-chain across 364 enumeration areas in Nigeria, and because there is an unmet demand for a detailed manual on designing and implementing a cold-chain system for a nutrition survey in resource-constrained settings, this paper documents the process using field experience from the NFCMS 2021.

MATERIALS AND METHODS

The methodology presented here is for designing and implementing a cold-chain system for a nutrition survey and was part of the implementation process for the NFCMS 2021. The NFCMS 2021 was a cross-sectional population-based survey. The methodology is as previously published [6]. The survey's primary objective was to identify key factors associated with poor nutrition in four target groups. The four target groups were children (aged 6-59 months), adolescent girls (aged 10-14 years), women of reproductive age (WRA), and pregnant women (aged 15-49 years). Anthropometry and micronutrient status were assessed in the four target groups. Dietary intake was assessed in children (aged 6-59 months), WRA, and



pregnant women (aged 15-49 years) only. Ensuring sample potency from the point of collection to the point of analysis was critical for the survey's success and called for a functional and efficient cold-chain.

A cold-chain is a chain of precisely coordinated events in temperature-controlled environments designed to store, manage and transport biological samples such as whole blood, serum, plasma, urine, and stool. Equipments such as cold boxes, freezer gel-packs, car freezers, dry-ice, and temperature data loggers are essential for maintaining cold-chain in the field. Facilities with refrigerators, -20°C , and -80°C freezers, referred to as cold stores, are also critical to supporting the cold-chain. In addition, three fundamental activities can make or break the functionality and efficiency of a cold-chain. They are the assessment of survey cold-chain requirements, the assessment and selection of cold stores, and the development of cold-chain logistics and plans.

Step-wise activities for designing and implementing a micronutrient survey cold-chain

The three key activities aforementioned were carried out to design and implement a functional and efficient cold-chain system for the survey. The cold-chain was to cover 364 survey enumeration areas (EAs) in all 36 states and the Federal Capital Territory in Nigeria.

1. Assessment of survey cold-chain requirements

Technical team meetings that included nutrition stakeholders in Nigeria and technical experts with a track record of conducting micronutrient surveys were held to design the cold-chain. The four objectives of the meetings were to:

1. Determine the types of micronutrients to be included in the survey
2. Document their corresponding sampling, analysis, and storage requirements
3. Determine equipment needed to support the cold-chain
4. Set the criteria for selecting functional cold stores

The Micronutrient survey manual [4] was used as a guide for assessing cold-chain requirements. Because folates are sensitive to temperature, oxygen, and light, their sample collection, processing, storage, and shipment dictated the cold-chain logistics for frozen samples. Serum folate is thought to be stable for a maximum of one week at 4°C and a maximum of one month at -20°C . For long-term storage, folate samples need to be stored at -70°C [7]. Therefore, the cold-chain plan was that after venous blood samples (~6 ml) were collected into the blue-top vacutainer in the field, samples would be centrifuged after at least 30 minutes and transferred



into two or three 2 ml labeled cryovials. The three cryovials were to be immediately put in cold boxes at -20°C . Within 4 hours of sample processing, the cryovials were to be transported by road to the nearest selected cold store (sub-collation site) and stored frozen at -20°C . After a maximum of 20 days and except for sub-collation sites in the vicinity of the International Institute of Tropical Agriculture (IITA), all samples were transported by air to the IITA human nutrition labs in Ibadan (central collation site) for storage at -83°C until they were ready to be shipped on dryice to international labs for analysis.

2. Assessment and selection of cold stores

An essential next step in implementing a cold-chain system for a micronutrient survey in a resource-constrained setting is identifying the locations where samples will be collected and determining the transportation and storage infrastructure available at each location. For the NFCMS 2021, it was determined that samples would be transported from the field to cold stores/sub-collation sites by road. The transit time was not to exceed 3 hours. This requirement necessitated a proximity analysis, mapping Local Government Areas (LGAs) of the 390 EAs to nearby LGAs with confirmed cold stores.

The survey planning team engaged relevant ministries at the federal and state levels to request the use of already existing cold stores. This was done concomitantly with the mapping of cold stores' proximity to selected EA locations. A database of the mapped cold stores was then generated. Contact details for the documented cold stores were extracted, and relevant individuals in charge of the facilities were contacted.

To support the selection of appropriate cold stores, a remote assessment of the cold stores in the vicinity of the NFCMNS 2021 EAs was done via telephone. A questionnaire (based on the set criteria for a functional cold store) for assessing the suitability of the identified cold stores was administered to the contact persons using the following 12 questions: (1) Which Local Government Area (LGA) is this facility in? (2) We have sent a letter to the Executive Secretary of the State Primary Health Care Development Agency, are you aware of this? (3) How many freezers are in your facility? (4) Can you dedicate an entire freezer for a period of 2 weeks to support the National Food Consumption and Micronutrient Survey? (5) Is there a generator for 24/7 operation? (6) Is this generator functional right now? (7) How much does it cost (in local currency) to fuel this generator for a 24-hour operation? (8) What is the power situation like at your facility? (9) Can you dedicate one individual to support this survey while we are at your facility? (10) Can we access the dedicated freezer after normal working hours and on weekends? (11) Does



your cold store have a storage facility where survey equipment and supplies can be kept short-term? (12) Can we use some part of this for storage of our supplies for a period of 2 weeks?

Based on the responses to the questionnaire, a long list of potential cold stores to be used by the NFCMS 2021 was prepared. A team of 42 assessors was trained in evaluating cold store suitability and deployed to the field to physically assess the longlisted cold stores and confirm their fit. Cold stores that passed the physical inspection of the set criteria for suitability were shortlisted and recommended for use. Consent letters from the Government authorizing the survey to use federal and state facilities were then sent to the shortlisted cold stores.

3. Development of cold-chain logistics and plans

During planning, it was noted that some critical supplies required a cold-chain during fieldwork, as shown in Table 1. Two cold-chains were required in the field for biological samples: +2°C to +8°C for whole blood; and -20°C for serum and plasma. Given Nigeria's relatively high ambient temperatures, all sample collection, handling, and processing were deemed time sensitive and to be carefully monitored. In addition, whole blood had a 48-hour time window within which to reach the analyzing lab in Lagos. Using simulations of different scenarios such as poor accessibility of EAs, limited access to electricity in the field, lack of air transport to move samples to a collation site, or a combination of these, the survey planning team developed context-specific cold-chain logistics by state, including a cold-chain preparedness plan. Field teams were given comprehensive cold-chain training on the cold-chain logistics, best practices for maintaining the cold-chain, monitoring temperature using temperature-monitoring tools, troubleshooting the cold-chain, and a communication plan. A dry-run of the cold-chain was piloted, and the experience was used to finalize cold-chain logistics and develop a cold-chain checklist.

The Micronutrient survey manual [4] was used as a guide for developing the NFCMS 2021 cold-chain logistics. Best practices from previous surveys referenced in the manual and recent large surveys conducted in the country, such as the 2018 Nigeria HIV/AIDS Indicator and Impact Survey (NAIIS), were adapted, and the resulting plan was documented in a survey field manual used to train the biomarker team. In the absence of a detailed manual with updated guidance on how to establish and implement a cold-chain system for a nutrition survey in resource-constrained settings, other knowledge and information gaps were filled by identifying potential sources of technical assistance or expertise that could be called upon as needed.



The logistics included:

Proper use of cold-chain equipment, including temperature monitoring tools and applications, proper use and storage of frozen gel packs, and proper storage of labeled specimens after sample collection and field-testing is complete

1. Proper management of samples in the field until they are processed
2. Monitoring the temperature of the cold box and portable freezer regularly to ensure the samples are stored at the correct temperature
3. A system for reporting and documenting any issues or incidents related to sample handling or storage.

These instructions were also summarized into checklists and a preparedness plan to support routine tasks and decision-making on critical tasks by the following:

1. Three field teams collecting biological samples in each of the six zones
2. Survey cold chain officer who had two key responsibilities. Daily aggregating samples from the biomarker teams at the EA level and transporting them to the appropriate cold stores for frozen samples and to the lab in Lagos for whole blood. And aggregating and accompanying the frozen samples in dry-ice every 25 days from the sub-collation sites to the collation site at IITA in Ibadan
3. Cold store personnel with the responsibility of maintaining the cold-chain at their facilities in the event of equipment malfunction, breakdown, or power outages.

In addition, the NFCMS 2021 cold-chain logistics included details on sample shipment to international labs in China, Germany, the United Kingdom, and the United States.

RESULTS AND DISCUSSION

1. Assessment of survey cold-chain requirements

Implementing a cold-chain system for a micronutrient survey in any setting should begin with determining the types of micronutrients to be included in the survey and their corresponding sampling and storage requirements.

The micronutrient component of the NFCMS 2021 had four target groups from whom biological samples were collected, as detailed in Table 1. Table 2 lists the



micronutrients of interest, their corresponding sampling, analysis details, and storage requirements identified by the planning team.

2. Assessment and selection of cold stores

A total of 365 facilities were identified nationwide, with 232 being near (up to 20 km away), 99 being far away (21-56 km), and 34 being very far away (57-184 km) from the EAs (Figure 1).

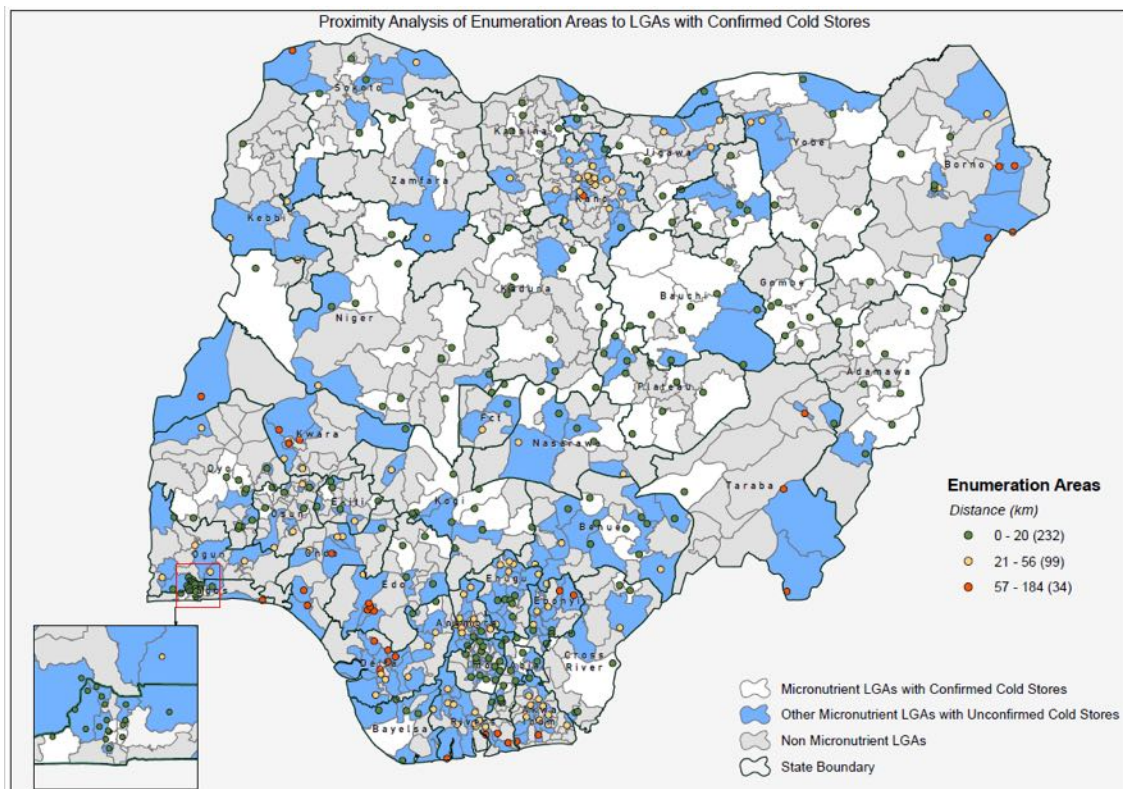


Figure 1: Map of Nigeria showing a proximity analysis of Local Government Areas (LGAs) of the 390 enumeration areas (EAs) of the survey to LGAs with confirmed cold stores

Of the 365 facilities mapped, 332 suitable cold stores were pre-selected based on the availability of dedicated personnel to support the survey beyond office hours, the availability of dedicated freezers for use by the NFCMS 2021, and the availability of a reliable backup power supply.

Following the remote assessment of the cold stores, a physical assessment of the 332 pre-selected facilities was conducted to validate the data generated. A total of 42 assessors (a supervisor for each zone of the six zones and one assessor from

each of the 36 states) who had been trained on the physical assessment of a suitable cold store and troubleshooting a cold-chain conducted the assessment.

The significant cold-chain challenges identified during the assessment were related to: (1) guaranteed access to power, particularly at night; (2) maintenance - shortage of qualified staff and spare parts; (3) potentially unreliable logistics arrangements with local airlines; and (4) the need to establish and implement controls, preferably using checklists, for compliance with NFCMS 2021 standards. Of the 332 facilities shortlisted, 292 facilities were successfully assessed.

About 70% of the store assessed had between one to three freezers, while the others had four to seven freezers. Of the 292 facilities assessed, only 235 facilities indicated their readiness to dedicate an entire freezer for two weeks to support the NFCMS 2021. Further assessment revealed that of these 235 freezers, 22 were not in optimal working conditions. Hence, 213 cold stores had acceptable freezers.

Among the 213 facilities with functional freezers, 144 had functional generators in good working conditions to run for 24 hours daily. Twenty five facilities had functional generators but could not operate them for up to 24 hours due to the absence of dedicated staff to run the generators overnight. Five facilities had no generators at all. Others had generators that were nonfunctional (13), or needed repairs/servicing (26) (Figure 2). The cost of fueling a generator for a 24-hour cycle was suggested at USD 25-30 per day. All facilities had trained personnel to oversee the cold store. The 144 facilities that met all the criteria for a functional cold store were selected as the go-to cold stores, while the 70 facilities, which met all other criteria but had challenges with the generators, were selected as backup cold stores.

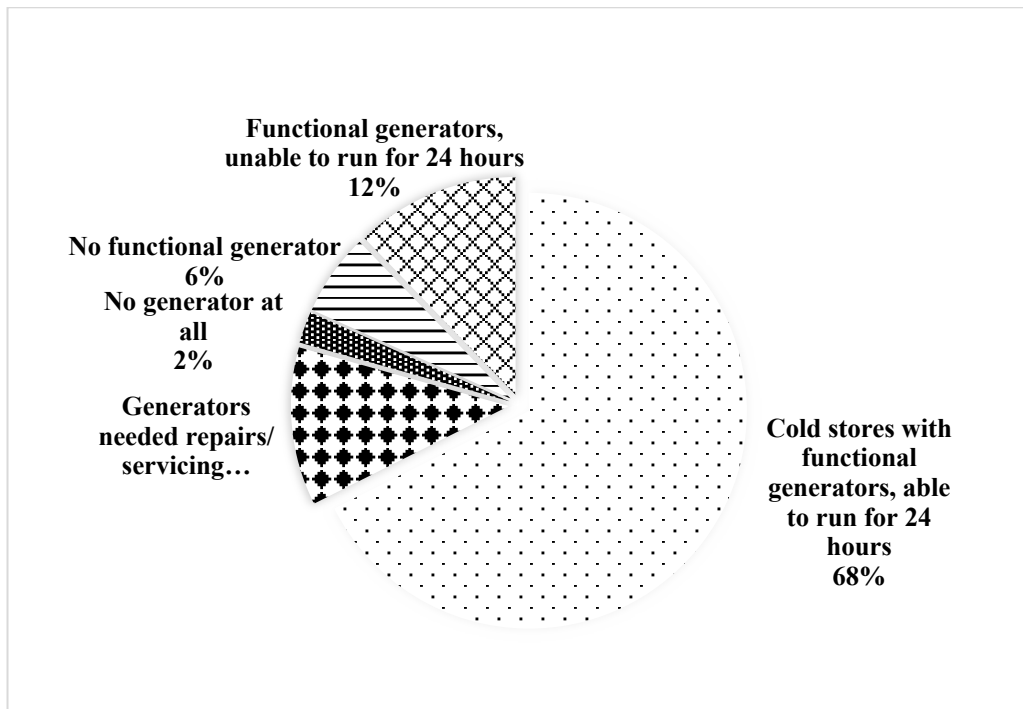


Figure 2: Availability of generators in pre-selected cold stores

3. Procurement of cold-chain equipment

The survey procured the following cold-chain supplies and equipment: power generator, portable car freezers, cold boxes, freezer gel-packs, dry-ice, temperature data loggers, shippers' boxes, dry-ice, -20°C and -80°C freezers. Procurement decisions for the cold-chain were based on the following information:

1. The proposed cold-chain plan for whole blood and frozen biological samples.
2. The proximity of enumeration areas to cold stores.
3. The frequency of sample movement from the field to sub-collation sites to the collation site.
4. Potential challenges with electricity and delays in hard-to-reach enumeration areas.

An innovation for this survey was using real-time temperature monitoring tools and a dashboard that allows for data visualization, such as charts, graphs, metrics, and even maps collected onto a single pane. An example of temperature data that can be linked to a dashboard is illustrated in Figure 4.

Most real-time temperature monitoring applications that incorporate Bluetooth technology for touchless temperature checks are inexpensive and easy to use. They can be a valuable tool in a cold-chain system for a micronutrient survey in resource-constrained settings as they ensure that the samples being collected and

transported are maintained at the appropriate temperature. This is critical for maintaining the integrity of the samples, as temperature variations can affect the stability of the micronutrients being measured. By using a real-time temperature-monitoring app, it is possible to continuously track the temperature of the samples and receive alerts if the temperature falls outside of the required range. This allows for immediate action to be taken to correct the issue and ensure the accuracy of the survey. In addition, real-time temperature monitoring apps linked to a dashboard accessible to all relevant stakeholders can also help to improve the efficiency and transparency of the cold-chain system by providing a clear record of temperature data. This data can be used to optimize the cold-chain system over time and ensure that the biological samples are being transported and stored most efficiently and effectively.

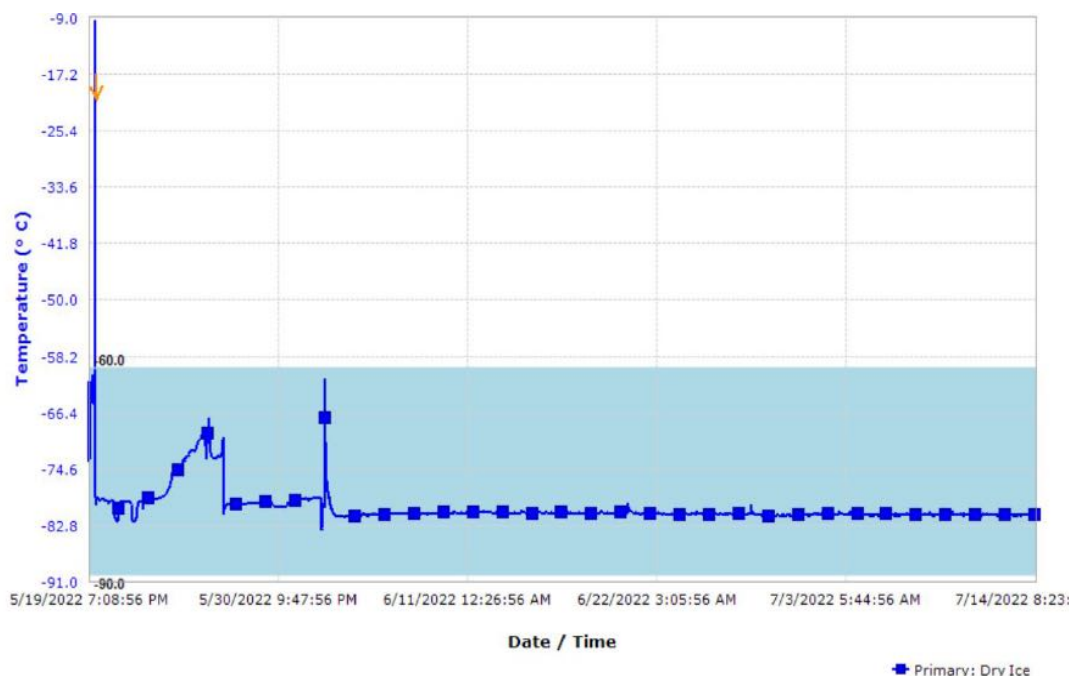


Figure 3: Snapshot of feedback from a real-time temperature-monitoring tool (TempTale Ultra BLE) showing temperature tracking of a frozen sample, in dryice, in transit

Development of cold-chain logistics:

Although it is not possible to predict all possible issues that can delay sample shipment, from our experience, the key challenges that a survey implementing team should prepare for include the following:

1. Procurement of dry-ice that can be plagued by unforeseen market shortages.

2. Understanding and aligning with the customs regulations of different countries was particularly challenging during the COVID-19 pandemic, with more restrictions and documentation required.
3. Restrictions on how much dry-ice airlines can carry, which in the case of a large survey like the NFCMS 2021, can lead to multiple shipments even to the same destination and add to the cost of the survey.

The lessons learned from transporting samples from the field to the local laboratory in Lagos and shipping samples from the central collation site at IITA in Ibadan to international labs (United Kingdom, Germany, United States of America, and China) can be summarized into two insights. For success, a survey should select reputable service providers with a track record of delivery and be sure to inspect-what-you-are-expecting religiously. Second, having detailed advance planning and creating value-based relationships with private sector partners can help avert any unforeseen crisis.

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

Implementing a cold-chain system for a micronutrient survey in a resource-constrained setting goes beyond developing a generic plan on paper for transporting samples from collection sites to analyzing laboratories using temperature-controlled equipment and relevant supplies. It is important to develop also contingency plans for handling unexpected delays or issues with sample transportation or storage. From our field experience in the NFCMS 2021, successful implementation and execution hinges on a detailed review and documentation of the cold-chain needs for the survey at the onset. In addition, key challenges that are likely to lead to significant delays during sample collection and handling, thus risking the integrity of the samples, must be explicitly identified and addressed in the cold-chain procurement plans and logistics. In the case of the NFCMS 2021, these included unreliable electricity, difficult terrain, and enumeration areas that were far from functional health facilities. Second, using already existing infrastructure, such as Government or privately-owned cold stores, will save costs but calls for extensive stakeholder engagement and collaborations with both the public and private sectors ahead of survey implementation. Third, a plan for evaluating the effectiveness of the cold-chain system and making necessary improvements is imperative. Leveraging technological advances to include cost-effective innovations such as real-time temperature monitoring tools with Bluetooth technology and dashboards will enhance the transparency, efficiency, and integrity of the cold-chain system guaranteeing the survey's success. Lastly, the survey identified an unmet demand for a detailed manual with



updated guidance on establishing and implementing a cold-chain system for a nutrition survey in resource-constrained settings.

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Table 1: Target groups and biological samples collected in the Nigeria National Food Consumption and Micronutrient Survey

Target group	Samples collected
Children aged 6 to 59 months (n= 6240)	Whole blood and stool
Adolescent girls aged 10 to14 years(n=1170)	Whole blood
Women of reproductive age, aged 15 to 49 years (n= 6240)	Whole blood, urine, and stool
Pregnant women aged 15 to 49 years(n=1170)	Whole blood, urine, and stool

Table 2: Nigeria National Food Consumption Micronutrient Survey (NFCMS) 2021 biomarker sampling, analysis details, and cold-chain requirements

Tests	Method of analysis/Equipment	Biological sample	Target group	Facility	Test location	Cold-chain requirements		
Haemoglobin	Anaemia was measured from whole venous blood sample using a HemoCue® 301 system.	Whole blood	Children (n= 6240) (C6-59m, 6-59 months)	Field mobile laboratory	On-site; EA location at primary healthcare facility or central location agreed upon by local leadership	+2°C to +8°C for HemoCue® 301 controls		
Malaria	Presence of Plasmodium falciparum malaria parasitemia in venous whole blood samples detected using a rapid diagnostic test kit (RDT)		Adolescent girls (n=1170) (ADOL, 10-14 years)			none		
Helicobacter pylori	The presence of IgG antibodies specific to H. pylori in venous whole blood samples was detected using a rapid qualitative immune assay test (RDT)		Women of Reproductive Age (n= 6240) (WRA, 15-49 years)			none		
Plasma glucose	Whole venous blood glucose concentration was measured using a HemoCue (Hb-201) instrument. Results were converted to equivalent plasma values using a constant factor of 1.11.		Plasma			Pregnant women (n=1170) (PREG, 15-49 years)	+2°C to +8°C for HemoCue® 201 cuvettes and controls	
Helminths	Presence of helminth eggs (Ascaris lumbricoides, Trichuris trichiura, Anclostomaduodenale, Necatoramericanus) in stool samples detected using microscopy	Stool	WRA (15-49 years)			Local ISO-certified laboratory facility	Lagos, Nigeria	none
Haemoglobin genotype	Whole venous blood was assessed using High-Performance Liquid Chromatography (HPLC)	Whole blood	C6-59m (6-59 months) WRA (15-49 years)					EDTA vacutainer sent to the lab in Lagos at (+2°C to +8°C); to reach within 48 hours
Glycated haemoglobin	HbA1c in whole venous blood sample assessed using a Bio-Rad D10 auto-analyzer	Whole blood	WRA (15-49 years)					



Iron status & markers of inflammation	Sandwich Elisa assay for Ferritin, serum transferrin receptors (sTfR), C-reactive protein (CRP), α1-acid glycoprotein (AGP) in serum	Serum	C6-59m (6-59 months) ADOL (10-14 years) WRA (15-49 years) PREG (15-49 years)	International laboratories	Germany	Samples are centrifuged in the field and immediately placed in cold boxes at -20°C; The serum is stored frozen at -20°C for up to 25 days and moved to -83°C until shipped on dry-ice to international labs for analysis.
Vitamin A status	Sandwich Elisa assay for Retinol-binding Protein (RBP) in serum	Serum	C6-59m (6-59 months) ADOL (10-14 years) WRA (15-49 years) PREG (15-49 years)		Germany	
	Serum retinol and MRDR in serum (20% sub-sample) was analyzed using HPLC and a standardized method for 3,4-di-dehydroretinol and retinol		C6-59m (6-59 months) WRA (15-49 years)		United States of America	
Folate status	Microbiological assay for serum folate;	Serum	ADOL (10-14 years) WRA (15-49 years) PREG (15-49 years)		China	
	Red Blood Cells (RBC) folate from whole venous blood lysate	Whole blood				
Zinc status	Serum zinc was assessed using Atomic Absorption Spectroscopy (AAS)	Serum	C6-59m (6-59 months) ADOL (10-14 years) WRA (15-49 years)		China	
Iodine status	Urinary iodine using ammonium persulfate	Urine	WRA (15-49 years) PREG (15-49 years)		China	
Vitamin B1 and B2 status	Erythrocyte transketolase (ETK) activity and Erythrocyte Glutathione Reductase (EGRAC) activity assay of saline- washed Red Blood Cells (RBC) in a 20% sub-sample	Whole blood	WRA (15-49 years)	United Kingdom		



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