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CHAPTER 2. METHODOLOGY

A two-stage cluster sampling design was applied with stratification to generate national and regional estimates. There are four regions in Papua New Guinea (Southern, Highlands, Mamose and Islands). The design of this survey does not allow for provincial estimates or any other categories other than regional (e.g. urban and rural). The recommendation to stratify the survey by region was based on the following assumptions:

- The diversity of the landscape, and agriculture and cultural practices may result in wide differences in the nutrition outcomes among the regions
- Programs may need to be introduced or targeted regionally. Region-specific estimates could help identify those regions in greatest need of interventions
- Not all nutritional interventions in Papua New Guinea are implemented nationwide and there are concerns that there could be significant regional variations

2.1 Sample size

The sample size for the PNG national micronutrient survey was determined using standard statistical procedures. The anticipated prevalence, desired precision, and assumed design effect for each outcome in each target group were determined based on the results from previous surveys and studies related to the outcomes of interest. Sample sizes for each outcome in each target group were calculated using the standard formula:

$$N = 1.96^2 \times \frac{pq}{d^2} \times DEFF$$

Where: N = Minimum sample size needed
1.96 = the z value to obtain a 95% confidence interval
p = the assumed prevalence of the nutrition outcome of interest in a target group
q = 1-p
d = the desired precision expressed as a half-confidence interval

DEFF = the design effect to account for the loss of statistical precision from cluster sampling

For many nutrition outcomes, conservative assumptions were made to intentionally overestimate the necessary sample size. The sample size is maximized if the prevalence is 50%. Similarly, design effects were overestimated to ensure adequate sample sizes.

For example, the calculation of a sample size for anemia in children 6-59 months of age was based on an estimated anemia prevalence of 50%, a precision of +/-10 percentage points, and a design effect of 2. Using the standard formula above, 192 children would be needed per region. The nationwide sample would require 4 times as many children 6-59 months of age because there are 4 strata, thus resulting in a total of 768 children. A certain proportion of selected households will be unavailable or refuse participation (household non-response) and a certain proportion of children in consenting households will be absent or their mothers will refuse consent for a finger stick (individual non-response).
Taking into account an estimated individual non-response of 20%, a household non response of 10%, and the proportion of children 6-59 months of age per household in PNG (0.7 children per household), the required number of households that need to be selected to obtain finger stick blood on 768 children is 1, 524. Table 2.1 shows a summary of the assumptions used to

generate the sample sizes for the major outcomes (See appendix 1 for a detailed description of the assumptions).

Table 2.1 Assumptions and estimated sample size for nutrition outcomes, Papua New Guinea National Nutrition Survey 2005.

Target group	Indicator	Estimated prevalence	Precision (Stratum-specific half CI)	*DEFF Assumed	Sample size needed (all 4 strata)	Individual non-response	No. per HH	Non response for HH	Total number of HH for 4 strata
House hold (HH)	Presence of iodized salt in the household	0.5	±12	4.5	1201	0%	1	10%	1,334
Children 6-59 months	Anemia	0.5	±10	2	768	20%	0.7	10%	1,524
	Iron deficiency	0.5	±10	2	768	20%	0.7	10%	1,524
	Malaria	0.5	±12	3	800	20%	0.7	10%	1,588
	Vitamin A deficiency	0.5	±10	2	768	20%	0.7	10%	1,524
	Wasting	0.1	± 5	1.5	830	10%	0.7	10%	1,463
	Stunting	0.5	±10	1.5	576	10%	0.7	10%	1,016
Children 24-59 months [a]	Hookworm	0.5	±15	3	512	30%	0.5	10%	1,626
Women 15-49 years	Iron deficiency	0.5	±10	2	768	20%	1.37	10%	779
	Anemia	0.5	±10	2	768	20%	1.37	10%	779
	Malaria	0.5	±12	3	800	20%	1.37	10%	811
	BMI <17	0.1	± 5	1.5	830	10%	1.37	10%	748
	BMI >25	0.5	±10	3	1152	10%	1.37	10%	1,039
	Urinary Iodine	0.5	±10	2	768	20%	1.37	10%	779
Men > 18 years	Anemia	0.1	± 5	1.5	830	25%	1.5	10%	820
	BMI < 17	0.1	± 5	1.5	830	10%	1.5	10%	683
	BMI > 25	0.1	± 5	1.5	830	10%	1.5	10%	683

* DEFF = Design effect (assumed for these calculations)

[a] Hookworm was only collected in children 24-59 months because children in this age group have greater exposure. It is also easier to collect stool samples from children this age

For most of the outcomes and target groups of interest, there are very few data on which to base the assumptions necessary to calculate sample size. As described above, a prevalence of 50% was selected for such indicators to provide the largest sample size for the given target population. For a given target group, sample sizes were calculated separately for each nutrition outcome measured in that group, and the maximum size was taken for that target group. It was decided that the number of households for the entire survey should be 1600 households, as this will provide at least the desired precision for most of the nutrition outcomes of interest. Table 2.2 shows the number of target individuals to be included in the sample of 1600 HHS.

Table 2.2 Expected number of participants by target group per strata and nationally

Target group	Indicators	Number of expected samples from participants per Strata	Total number of participants nationally (4 strata in total) 1600 households
Children 6-59 months	Laboratory	202	808
	Anthropometry	227	908
Women 15-49 years*	Laboratory	197	788
	Anthropometry	222	888
Adult men 18 years and above*	Laboratory	203	812
	Anthropometry	243	972

* On ½ of all households. These figures take into account the expected presence of participants at the household and the household and individual response rates

At every household visited, anemia, iron deficiency, vitamin A deficiency, acute phase proteins, malarial load, wasting, underweight and stunting were assessed for each eligible child in the household. In all households with a child aged 24-59 months, hookworm was also measured. In every second household, anemia, iron deficiency, BMI, urinary iodine and malarial load was measured in all non-pregnant women of child-bearing age. In every second household, anemia was measured in all men above 18 years of age. See appendix 2 for the design effects (DEFF) for the major indicators.

2.1.1 First stage sampling

The National Statistical Office provided a list of all census units in PNG in an Excel spreadsheet created during the 2000 census. For each of the four regions a list of the number of households in each census unit, and a column of cumulative sums was created. In the first sampling stage, 25 primary sampling units (PSUs) were selected from this list for each region. Sampling probability proportional to size was done by calculating a sampling fraction (the total number of households in each of the four regions divided by the number of required PSUs [25]), and adding this sampling fraction repeatedly to an initial random number. Each census unit in which the resulting number fell became the site for one PSU. No census unit was selected more than once. If the census unit selected had fewer than 25 households the next nearest census unit was selected and combined with the original census unit. Appendix 3 shows a list of the census units selected. Final data was collected from 97 clusters. Three clusters were inaccessible (8, 58, 87) at the time of the survey due to logistical constraints. Despite efforts to reach them on several occasions data collection activities in these three clusters was aborted.

The 100 randomly selected PSUs are located in all 20 provinces and in 75 of the 87 districts in PNG: 16 districts in Southern region, 24 in the Highlands regions, 23 in the Mamose region and 12 in the Islands region. Although all the provinces were included in the survey, it is important to note that the precision around the estimates of the prevalence of all outcomes is not sufficient to interpret the results of the survey by province.

2.1.2 Second stage of sampling

In each PSU the survey team worked with the local leaders and the community to create a household listing of all households in the selected PSU. The households on the list were then numbered, and 20 households were selected using a random number table. In each PSU it was confirmed that all households currently residing in the PSU were included on the list of households. Any households missing on the list, such as recent returnees, were added to a preexisting list. In large primary sampling units (greater than 250 households), where it was not possible to make a list of all households, a map of the area was drawn and split into segments. The segments were approximately the same size and geographic boundaries such as roads, rivers, buildings and important landmarks were used to identify the boundaries of one segment from another. The number of households in each segment was listed and a list of cumulative sums was created. A segment of the PSU was then chosen at random, probability proportional to size. The households within the selected segment were then mapped and 20 households were selected. In situations where the selected PSU had fewer than 20 households the team leader combined the selected PSU with the closest neighboring census unit and made a complete listing of all the households in both the PSU and census unit.

In each selected household, all eligible persons in the identified target groups were asked to participate in the survey. For the purposes of the PNG national micronutrient survey, a household was defined as a group of people who share a common cooking pot and shared household resources, such as food and bedding. Members of a household were not necessarily relatives by blood or marriage.

All children 6-59 months of age were selected in every household included in the survey. Every non-pregnant woman aged 15-49 years and all men aged 18 years or older were invited to participate in the survey in every other household. Table 2.3 show the number of survey participants from each target group that participated in the survey.

Table 2.3 Number of survey participants, by region and nationally, PNG National Nutrition Survey 2005

	Households	**Children (6-59 months)**	**Pregnant women of childbearing age (15-45 yrs)***	**Non-pregnant women of childbearing age (15-45 yrs)**	**Men (18>yrs)**
Region					
National	1403	937	64	783	804
Southern	342	226	16	258	213
Highlands	359	209	19	181	212
Mamose	354	255	15	176	197
Islands	348	247	14	168	182

* Pregnant women were not a target group in the survey. Some of the women who were interviewed during the survey were pregnant. Thos women completed the survey questionnaire but were not asked to provide any biological samples or be weighed and measured.

2.2 Ethical considerations

The ethical and technical considerations of the survey were extensively reviewed. The survey methodology was approved by the Medical Research Advisory Committee of Department of Health. The right of individuals to choose to participate or not to participate was ensured and respected. Informed consent (see Appendix 4) was obtained from each adult participant and primary care giver of children asked to participate in the survey, once the purpose and content of the survey had been explained. Consent was obtained verbally and the interviewer indicated on the top of the participant form if consent was obtained or not. Participants were also informed that they were free to refuse at any point during the survey. The hemoglobin test result of each subject was provided and the subject was referred to the health clinic if the results indicated moderate to severe anemia as defined by WHO (WHO 2001).

2.3 Survey Teams, training and implementation

2.3.1 Survey teams

Six survey teams were recruited. Both a male and female members were assigned to each team. Each team was made up of 4 individuals as follows:

- One team leader
- One interviewer and anthropometry assistant
- One anthropometrist
- One laboratory technician

Where possible, cars were provided for the teams and the driver assisted teams where needed. For many of the clusters, the routes were impassible by car and local people were hired to assist with carrying equipment to the cluster. In each district, focal persons were identified and assigned by the Department of Health to assist teams. Focal persons provided the teams with assistance in locating the correct primary sampling unit, helping the team access the PSU, collecting specimens and data collection forms completed in the PSU and transporting them to Port Moresby. They also provided an essential link in the communication between the survey supervisors located in Port Moresby and team members and helped troubleshoot any problems in the field. The survey task force led by the survey coordinator supported and guided the teams in technical, logistical and managerial aspects throughout the data collection period. The CDC technical team was based in Port Moresby for the planning, training, and initial implementation phase of the survey. They provided intensive supervision and quality control for the collection of data in the Port Moresby PSUs. Once the survey was underway they returned to Atlanta and provided close support via telephone and email.

Team member selection began with an interview process by DOH/UNICEF. Most of the survey team members were staff from the Department of Health or were university students. Many of them had some experience working with NGOs or experience working in health care settings. It was required that each survey team included at least one male to ensure the security of the female team members.

Thirty one potential team members, selected from the interview process began training for the survey. After one week of training, 24 survey team members were selected from the 31 training participants based on the results of a written test, previous experience and performance during the training. In each team one person was selected as the team leader. Team leaders were

chosen based on skills and leadership qualities. The team leaders participated in an additional day of training. The survey pilot test proceeded with only the selected team members.

2.3.2 Training

A two-week training program was conducted to prepare the survey teams. Three technical advisors from CDC, with assistance from members of the University of Papua New Guinea and the Department of Health coordinated the training. The training was conducted in English. The training included lectures, PowerPoint presentations, practical exercises and role plays. The training covered aims and objectives of the survey, survey methodology, team composition, team and individual responsibilities, field procedures, selection of households and eligible participants, interview techniques, questionnaire administration, anthropometry, and blood, urine, stool and salt sample collection, storage, and transport guidelines.

Data collection forms were created after consultation with national and international organizations providing nutrition and health services to the population of Papua New Guinea. The types of data collected conform to the types of data that have previously been collected in Papua New Guinea. The data collection forms were first developed in English. Written translations were made into Pidgin and checked by back translation. All data collection forms can be found in Appendix 5. The survey instruments were pre-tested in Geruhu, a suburb of Port Moresby and Kalo village in Central Province. About 25 households in both locations participated in the pre-test. Neither of these sites was included in the survey and revisions to the procedures and data collections instruments were made based on these pretests.

Laboratory technicians in the teams were trained by a CDC laboratory specialist on the following:

- Correct techniques for finger puncture and capillary blood collection into microtainers;
- The use of field instruments (Hemocue) to measure haemoglobin;
- Monitoring of quality control when using the Hemocue
- Processing and storage of dried blood spots (DBS) for the analysis of retinol binding protein (RBP), transferrin receptor(TfR), acute phase proteins AGP and CRP;
- Monitoring of humidity for proper storage of DBS in the field
- Preparation of malaria slides;
- Collection, storage, and transport of urine and stool samples
- Collection, storage, and transportation of salt samples

The training concluded with a two-day pilot survey conducted in Hanuabada village in Port Moresby. Hanuabada was not included in any PSUs selected for the actual survey. The pilot simulated the surveying of one cluster by each team and was conducted under close supervision of senior technical advisors from CDC, the Department of Health, the University of Papua New Guinea and UNICEF. The pilot testing was followed by a day of organized feedback that addressed and resolved various technical and logistical issues.

2.3.3 Survey implementation

Following PSU selection, the Department of Health in Port Moresby sent letters to Provincial Health Advisors (PHA) informing them about the survey, the PSUs that had been selected, and the dates for data collection. The PHAs in turn informed the District Administrators of the selected districts of the PSUs to be assessed and dates of the survey. They also helped the survey coordinator to identify suitable focal points in the districts to assist teams with the survey.

All relevant opinion leaders at provincial and district level, such as community health workers, were identified by the Department of Health and UNICEF and were acquainted with the survey objectives and implementation plan. These leaders were requested to encourage the population to participate in and support the survey. A formal request was also issued by the Department of Health to regional, provincial and district level leaders request their cooperation and support during the survey.

Before traveling to a selected census unit (village), the survey team first visited the respective main provincial centre to be introduced to the provincial administrator, PHA and health officials in the selected districtsdistrict governor and district health administration. They also met the focal person designated to assist the team during the course of the survey in the area. The team leader further described the aim, purpose and methodology of the survey and answered any questions the local authorities had.The focal person accompanied the team to the census unit in some circumstances.

Once in the PSU (village), the team contacted the village leaders and delivered the letters from the provincial and/or district officials. The team leader reiterated the objectives and procedure of the survey including the information that would be collected and how they would collect it. Once permission had been granted by the village leaders for the survey to go ahead a list was made of all the households in the census unit. Households to be surveyed were identified by selecting 20 households at random from the list using a random number Table.

A household was defined as a group of people who sleep and eat together on a regular basis. Household members didn't need to be living in the same room. However, if they shared their meals "from the same pot", they were considered members of the household. Families who lived in the same room with or without partition, but took their meals from separately prepared pots, were regarded as different households.

The head of a household was defined as the person in the household who makes the major decisions for all the household members, such as financial expenditures, schooling, medical care and food. If the household head was not at the house during the time of the survey, the individual with the household responsibilities while she or he was away was considered the 'acting' household head. If the household head had recently died, the spouse, parent, brother, or elder son of the deceased was considered the household head, depending on arrangements in each family.

Upon arrival at the selected households, the team leader usually accompanied by community leaders or representatives, explained to the head of the household the purpose and procedures of the survey and how the household was selected.

Each team conducted their first cluster of data collection in the Port Moresby area. This allowed for close supervision and support by the CDC technical team, and enabled quick response to unforeseen problems before the teams traveled to remote areas. A full day review session was organized following the completion of the Port Moresby clusters, prior to the teams traveling outside the capital city.

2.3.4 Interview procedure

With the permission of the head of the household, the interviewer, anthropometrist and lab technician entered the household. The interviewer made further introductions of the survey to prospective participants and began the interview process. First, the household head was asked

questions about the members of the household and their ages, the kind of facilities available to the household and also questions about the consumption of various centrally processed foods. A local calendar of events created by the team was used to determine ages of children living in the household. Local calendars were constructed by the team as needed because of different local events that have occurred in different parts of the country.

Before proceeding with individual interviews, informed consent was obtained from each participant, or in the case of children, their primary caretakers. Each participant was administered the appropriate questionnaire corresponding to the target group. Information on children under the age of five years was obtained through an interview with the primary caretaker. Where possible the interviews were conducted in Pidgin. When that was not possible, and none of the team members had experience in the local language, a local translator was used. One of the reasons for keeping the data collection form very simple was to avoid difficulties translating questions or responses. Local leaders and focal points accompanying the teams assisted with translation if needed. In addition to interview questions, anthropometric measurements, blood and urine were collected from the appropriate target groups. Table 2.4 details the indicators assessed for each target group.

Table 2.4 Target groups and indicators assessed, National Nutrition Survey, Papua New Guinea 2005

Target group	Specimen Collected or Measurement Taken	Indicators Assessed
Children (6 – 59 months)	Blood	- Hemoglobin - Retinol binding protein (DBS method) - Transferrin receptor (DBS method) - C- reactive protein (DBS method) - AGP (DBS method)
	Height and Weight	- Anthropometric status
Children (24-59 months)	Stool	- Hookworm egg count
Women (15 – 49 years)	Blood	- Hemoglobin - Retinol binding protein (DBS method) - Transferrin receptor (DBS method) - C- reactive protein (DBS method) - AGP (DBS method)
	Urine	- Urinary iodine
	Height and weight	- Anthropometric status
Men (18 ≥ years)	Blood	- Hemoglobin
	Height and weight	- Anthropometric status
Household	Salt	- Iodine content

In each household selected, the household head was asked questions about the household and current members of the household. In addition, a sample of each type of salt from each selected household was collected, if available, to be tested for iodine content.

In households where women were selected to be interviewed, survey workers asked each woman of reproductive age questions regarding night blindness, whether she used tobacco, whether she slept under a mosquito net, her last pregnancy and information about that child,

such as the child's birth weight. Women who had given birth during the past three years prior to the survey were asked to recall the birth weight of her last born child. Where possible women were asked to show any documentation where the birth weight was recorded. Women were asked if they were currently pregnant, if thery were they were only asked to complete the questionnaire. Non-pregnant woman had their height and weight measurements taken, a urine sample collected and blood collected to test for Hb and to prepare dried blood spots and a malaria slide.

In households where men were selected to be interviewed, survey workers asked questions to each man regarding his educational status, tobacco usage and mosquito net usage. In addition, each man had his height and weight measurements taken and a finger prick conducted to assess his hemoglobin level.

Information was gathered from an adult household member, preferably the mother, on each child less than 5 years of age regarding mosquito net usage, breastfeeding history, vitamin A supplementation and introduction of complementary foods. Where available, baby clinic books were used to identify the child's correct age and record the date the child received his/her last vitamin A supplement. If baby clinic books were not available, then the age of the child was determined using mothers recall and a local calendar. Survey workers then took a sample of blood and tested the child's hemoglobin, prepared dried blood spots and a malaria slide. Mothers were asked to collect a stool sample from her child if the child was aged between 24-59 months of age. If the specimen could not be collected at the time of the visit, then the survey workers left a collection cup and returned the next day to collect the specimen. Survey workers also measured the child's weight and height.

Children less than 5 years of age, women of reproductive age and men were weighed to the nearest 100 grams with the Seca Uniscale. For children less than 24 months of age, length was measured to the nearest millimeter in the recumbent position using a Shorr height board. Children 24 months of age or older were measured in a standing position using the Shorr board. Women's and men's height was measured using the Shorr board with the extension piece added.

Pre-printed labels were used to identify completed survey questionnaires and the associated biological and salt samples collected for later testing. Details of the labeling procedure are provided in Appendix 6.

When no-one was present in a survey household, the team revisited the household up to three times before declaring a non-response. If an eligible child, woman or man was absent when the survey team visited the household, the household was re-visited up to two times, before declaring the participant absent.

On average it took 4-5 days to complete data collection in each cluster. Travel between clusters took at least 1-2 days.

2.3.5 Anthropometry

Heights and weights of children aged 6 – 59 months, non-pregnant women 15-49 years and men $\geq$18 years were measured using standard methods[2] described in Appendix 7. Individuals were excluded if they had a disability that prevented them from standing up straight or lying down flat, were wearing casts or heavy bandages or if they were missing one or more limbs.

The indices for interpreting pediatric and adult anthropometric data are provided in Tables 2.7 - 2.9.

a) Age

When the date of birth was known, the age of preschool children was calculated based on the difference between the birth date and the date of the measurement. Verification of birth date by baby clinic book or other written record, for example baptism certificate, was made when possible. When the birth date of the child was unknown, a local event calendar (which included key religious and national events during the previous six years) was used to estimate the child's age. Women's and men's ages (in years) were based on self-report and without the use of a local event calendar.

b) Length/height

For children less than 24 months old, recumbent length was measured to the nearest 0.1 cm using a Shorr board. The same device was used to measure standing height to the nearest 0.1 cm for children >24 months. Adult women and men were measured to the nearest 0.1 cm using a Shorr board with the adult extension piece attached.

c) Weight

Seca Uniscales were used to measure weights of preschool children, women and men to the nearest 0.1 kg. Children who were too young to stand on their own were weighed while being held by their mothers (or an adult) using the mother-child tare function on the scale.

2.3.6 Blood collection - capillary sampling, processing and testing

Capillary blood was collected via finger puncture from the middle or ring finger using semi-automated lancets with 2.25 mm needles. Blood was collected into a microtainer as described in Appendix 8. If unsuccessful on the first attempt, a finger puncture was attempted a second time. For each participant, between 250-500µL of blood was collected. Once the microtainer was filled, the microtainer was inverted ten times. The blood was then used to assess hemoglobin status and prepare malaria thick smears and dried blood spot cards (see procedures below). Blood collection materials were disposed in biohazard bags and taken to the district/provincial Department of Health biohazard waste management for incineration.

a) Hemoglobin measurement

Hemoglobin (Hb) concentration was determined in the household using the HemoCue system (Angelholm 2005). Quality control of each HemoCue instrument was performed every morning of data collection, using a control cuvette to ensure that the Hemocue optics were functioning properly (Appendix 9a and b). Three different levels of liquid controls (high, medium and low) were also tested daily to ensure the integrity of the microcuvettes. If the Hb value was outside the expected range the Hemocue quality control was performed to ensure that the techniques being used and the equipment were accurate. If the accuracy reading was outside the range, of the control cuvette and the Hemocue had been cleaned, then the instrument was replaced.

Anemia was defined as low Hb concentration using World Health Organization criteria (WHO 2001) (see Table 2.13). Participants were informed whether their hemoglobin level was normal or low. If their hemoglobin was low they were referred to the nearest health clinic, (see appendix 10 for the team referral sheet and appendix 11 for the referral slips).

b) Malaria slide

A malaria thick smear was created for all children and women who participated in the survey and provided a blood sample. Using the 25 µL pipette tip, a drop of blood (approximately 12 µL) from the Microtainer was placed onto the slide. The drop of blood was spread into a circle about 1cm in diameter. The slide was then placed in the box and left until the smear was semi dry and the box could be shut (appendix 8). The slides were then sent to Port Moresby where they were read at the University of Papua New Guinea. The malaria prevalence of the initial analysis was very low and it was decided that the slides should be re-read. All of the slides were re-read in Papua New Guinea and the data were analyzed. The difference in the prevalence of malaria from the two sets of analysis was extremely large (there was a 58.7 percentage point difference for the national prevalence of malaria). A report comparing the data from the two analyses looked at malaria severity and malaria in relation to altitude and anemia. Based on the extreme differences in the data, and the lack of a relationship between malaria and altitude and hemoglobin it was decided to exclude the malaria data from this report.

c) Dried blood spot (DBS) collection, processing and testing

In this survey, dry blood spots (DBS) were used as it is the best method under field conditions that does not require centrifugation, freezing or transport of blood samples in a cold chain.

After the hemoglobin was tested and the malaria thick smear prepared, dry blood spots (DBS) were prepared by transferring the remainder of the blood in the microtainers to pre-printed circles on filter papers using a 25 µL calibrated micropipette. As many spots as possible were filled on the DBS cards. If blood was left in the microtainer after all spots had been filled, 25 µL spots were pipetted in the spaces between the circles taking care not to allow the spots to overlap. The DBS card was then transferred to cardboard racks that were specially designed for drying DBS-filter papers. The box holding the DBS card was left open during the remainder of the stay in the household and a small hand fan was positioned approximately 20 cm away form the box to help the card dry. The box was closed before being transported to the next house.

Each evening, after the spots were completely dry, the DBS cards were packed in low gas permeable bags, with each filter paper separated by glassine (weighing) paper and along with desiccant packs and humidity indicator cards. Each gas permeable bag was then put in pleated bags (Tyvek bags), for storage/shipping. For the duration of storage, humidity indicator cards were monitored every day and desiccants packets were changed as necessary. The DBS cards were sent to Port Moresby where they were packed in dry ice and shipped to the laboratories at CDC. From CDC they were re-packaged and sent to SEAMEO-TROPMED laboratory in Jakarta, Indonesia, for testing of transferring receptor (TfR), retinol binding protein (RBP), C-reactive protein (CRP) and Alpha 1-acid glycoprotein (AGP).

In addition to the dried blood spots, venous blood samples were collected from a small subsample of participating non-pregnant women and children 6-59 months of age to calculate a correction curve needed for the RBP analysis.

2.3.7 Urine collection, processing and testing

Urine samples were collected from non-pregnant women according to specific procedures (Appendix 8). The lab technician pipetted equal amounts of urine (1.5 mL) from the cup into two pre-labeled iodine-free cryovials using a disposable pipette. The urine specimens were then stored in a cryovial box until they could be sent to Port Moresby. Once the samples arrived in Port Moresby they were kept frozen at <-20 ^{o}C. Urine collection caps and pipettes were disposed of in biohazard bags and taken to district/provincial MOPH biohazard waste management for incineration. The specimens were analyzed at the University of Papua New Guinea.

2.3.8 Stool testing

All eligible children 24-59 months were asked to provide a stool sample to look for the presence of hookworm infection. If the child was unable to provide a sample during the household visit, a container was left with the child's primary caretaker so that a specimen could be collected. Field workers arranged to re-visit the household the following day to collect the specimen. Using a wooden specimen stick, a small amount of stool (the size of a grape) was transferred to the specimen tube, which contained fixative. The cap was tightened and Parafilm was wrapped around the cap. The tube was then shaken vigorously and placed in a Ziploc bag before being transported to Port Moresby. The stool specimens were analyzed at the University of Papua New Guinea. A 10 % sub sample of the stool specimens was also analyzed at CDC.

2.3.9 Salt sample collection, processing and storage

The characteristics of household salt were assessed according to the type of salt (e.g. course versus fine), commercial producer (e.g. brand of salt), country where the salt was produced, the country where it was packaged and whether or not the salt was labeled as iodized.

Within each household, the household head was asked if a sample of each type of salt in the household could be taken from the household and tested. Households that provided a salt sample for quantitative analysis were given a replacement bag of national brand iodized salt.

Approximately four tablespoons of salt was collected from each household in a plastic Ziploc bag, and was sealed and labeled for transport to Port Moresby for quantitative analysis using the single wave length WYD Iodine Checker (Salt research institute 2006). Salt preparation and analysis was conducted according to the instrument manufacturer's procedures and was monitored by the Laboratory trainer from CDC.

If there were two types of salt in the household a sample of the second type of salt was also collected.

2.4 Data collection, entry and analysis

2.4.1 Data entry

A computer database, using CSPRO 3.1, was developed by a local consultant hired by UNICEF in Port Moresby to facilitate the entry of the survey data. Minimum and maximum allowable values, specified numbers of digits for entry, and skip patterns were embedded into the data entry screens to minimize data entry errors. The data were entered twice by trained university students at the University of Papua New Guinea, and the data files were compared electronically to correct any data entry errors.

Laboratory tests were single entered into Excel spreadsheets by each testing laboratory. Data in these spreadsheets were cleaned and merged with the questionnaire data, by individual or household ID numbers, for analysis.

2.4.2 Data analysis

Data analyses were performed using SPSS Version 13.0 (SPSS, Inc., Chicago, USA) and Epi Info Version 3.3.2, October 5, 2004 (CDC, Atlanta, USA).

Cutoffs to define vitamin and mineral deficiencies and their level of public health significance were based on WHO, UNICEF, CDC and INACG recommendations (Table 2.5). Cut-offs for anthropometry indicators used the new WHO growth standard (De Onis et al 2008).

Table 2.5 Biochemical indicators and cutoffs used to identify nutrition status within target group, National Nutrition Survey, Papua New Guinea 2005

Nutrition status	Indicator	Target group		
		Children (6-59 months)	**Non-Pregnant women (15-49 years)**	**Men (18> years)**
Iodine deficiency	Urinary iodine (UI)	Not tested	International cutoff not agreed upon. For this survey a median UI <100 µg/L was used as an indictor of moderate deficiency in the population	Not tested
Vitamin A deficiency	Retinol binding protein (RBP)	≤ 0.35 umol/L severe deficiency ≤ 0.70 umol/L deficiency	≤ 0.35 umol/L severe deficiency ≤ 0.70 umol/L deficiency	Not tested
Anemia	Hemoglobin (Hb)	<11.0 g/dL	<12.0 g/dL	<13.0 g/dL
Iron deficiency	Transferrin receptor (TfR)	>8.0 µg/l	>8.0 µg/l	Not tested
Iron deficiency anemia	Elevated TfR and low Hb	Hb<11.0 g/dL and TfR>8.0 µg/l	Hb<12.0 g/dL and TfR>8.0 µg/l	Not tested

2.4.3 Statistical weighting

Statistical weighting was used in the analysis because the actual number of households surveyed between regions was variable; therefore weighting at the regional level was required to calculate national estimates.

2.4.4 Anthropometry analysis and interpretation

a) Infants

Stunting, underweight, and wasting, were assessed in children using the new WHO 2006 growth standards (De Onis et al 2008). The results from the new WHO standards are presented in the body of the report. Information on anthropometric indices is presented in Table 2.6.

Table 2.6 Anthropometric indices

Type of malnutrition	Anthropometric index	Degree of malnutrition	Definition using z-score
Acute	Weight-for-height	Moderate	≥ -3.0 but < -2.0
		Severe	< -3.0 or edema
		None	≥ -2.0
Chronic	Height-for-age	Moderate	≥ -3.0 but < -2.0
		Severe	< -3.0
		None	≥ -2.0
Underweight	Weight-for-age	Moderate	≥ -3.0 but < -2.0
		Severe	< -3.0
		None	≥ -2.0

The anthropometric indicators length/height-for-age, weight-for-age, weight-for-length/height were determined for children surveyed using the WHO Anthro package. Values outside the following ranges were excluded from analyses as recommended by WHO:

Weight-for-Height Z-score (WHZ) <-5.0 or >5.0
Weight-for-Age Z-score (WAZ) <-6.0 or >5.0
Height-for-age Z-score (HAZ) <-6.0 or >6.0

At the population level, the prevalence of low Z-scores for each anthropometric index is categorized according to prevalence and public health significance (Table 2.7).

Table 2.7 Relative prevalence of low anthropometric values (WHO 1995)

Anthropometric Index	Low	Medium	High	Very High
Moderate WHZ	<5.0%	5.0-9.9%	10.0-14.9%	≥15.0%
Moderate HAZ	<20.0%	20.0-29.9%	30.0-39.9%	≥40.0%
Moderate WAZ	<10.0%	10.0-19.9%	20.0-29.9%	≥30.0%

b) Adults

Body Mass Index (BMI) was calculated for non pregnant women and men as weight (kg) divided by height (m) squared (wt[kg]/ht $[m]^2$). Malnutrition was assessed using WHO-recommended categories for BMI (WHO 1995).

BMI is used to classify an individual as underweight, normal weight, overweight or obese (see Table 2.8).

Table 2.8 BMI categories, National Nutrition Survey

BMI	Category of malnutrition
< 16.0	Severe thinness
16.0 – 16.9	Moderate thinness
17.0 - 18.4	Mild thinness
18.5 - 24.9	Normal
25.0 - 29.9	Overweight
≥ 30	Obese

Women who were pregnant at the time of the survey did not have their height and weight measured or blood and urine specimens collected.

The public health significance of the population prevalence of low BMI (<18.5) is presented in Table 2.9. A high prevalence of low BMI may be an indication of food insecurity and/or widespread disease. Excessive thinness may also highlight the vulnerability of populations living in difficult circumstances such as seasonal variation, drought or epidemics (WHO 1995).

Table 2.9 Categories of prevalence of low BMI (<18.5) according to public health significance (WHO 2008)

Normal	Low Prevalence (warning sign, monitoring required)	Medium Prevalence (poor situation)	High Prevalence (serious situation)	Very High Prevalence (critical situation)
3-5%	5-9%	10-19%	20-39%	≥40%

2.4.5 Urinary iodine analysis (UI) and data interpretation

Urinary Iodine (UI) concentration is the recommended biochemical index of choice for evaluating the degree of iodine deficiency and for assessing the impact of deficiency control programs. When appropriate sampling procedures are used, the UI concentration in casual urine samples collected from either children (6 – 12 years) or non-pregnant women can provide an adequate assessment of the iodine nutrition status in the population. The UI concentration in an individual can vary within a day and from day to day. This variation tends to even out among populations. Thus, the population distribution of the UI concentration is important, rather than individual UI concentrations. The UI concentrations obtained from populations are usually not normally distributed; the median (rather than the mean) is therefore used as a measure of central tendency. In addition, percentiles rather than standard deviations are used as measures of spread.

For this survey Iodine deficiency was based on low urinary iodine (UI) level in a casual urine sample using Method A with ammonium persulfate (WHO 2001). Specimens were analyzed at

the University of Papua New Guinea. Iodine status was only assessed for women of child-bearing age (15-49 years) as the survey was a household survey and school age children would be harder to access at the household.

The interpretation of a population's iodine status based on median urinary iodine levels is presented in Table 2.10.

Table 2.10 Epidemiological criteria for assessing a population's iodine status based on median urinary iodine concentrations

Median urinary iodine (ug/l)	Iodine intake	Iodine Status
Non-pregnant women		
< 20	Insufficient	Severe iodine deficiency
20-49	Insufficient	Moderate iodine deficiency
50-99	Insufficient	Mild iodine deficiency
100-199	Adequate	Adequate iodine nutrition
200-299	Above requirements	May pose a slight risk of more than adequate iodine intake in these populations
≥300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)

2.5.6 Salt

The World Health Organization guidelines state that for successful elimination of iodine deficiency at least 90% of households should have access to, and regularly use, adequately iodized salt (≥15 parts per million [ppm] iodine content) (WHO/ICCIDD/UNICEF 2007).

2.5.7 Anemia

Anemia, defined as low Hb, is often used as a proxy indicator of iron deficiency. The Hb cut-offs for anemia, based on age and sex, are presented in Table 2.5. Hb values <4 g/dL or >18 g/dL were considered extreme and excluded from the analysis.

To determine the prevalence of anemia, the individual observed Hb concentrations were adjusted based on altitude, and cigarette smoking, according to WHO/UNICEF/UNU 2001 and INACG 2004 recommendations (Table 2.11).

Table 2.11 Adjustments to observed Hb values based on altitude, and cigarette smoking (WHO/UNICEF/UNU 2001, Nestel 2004 and CDC 1998)

Condition	Hemoglobin Adjustment (g/dL)
Altitude (m)	
m < 1000	--
1000≤ m <1250	-0.2
1250≤ m <1750	-0.5
1750≤ m <2250	-0.8
2250≤ m <2750	-1.3
2750≤ m <3250	-1.9
3250≤ m <3750	-2.7
3750≤ m <4250	-3.5
4250≤ m <4750	-4.5
4750≤ m <5250	-5.5
M ≥ 5250	-6.7
Cigarettes smoked per day	
Fewer than 10 cigarettes/day	--
10 ≤ cigarettes/day < 20	-0.3
20 ≤ cigarettes/day	-0.5
Smoker, amount unknown	-0.3

The public health significance of anemia, based on prevalence of the indicator, is presented in Table 2.12.

Table 2.12 WHO classification of public health significance of anemia in populations based on the prevalence of anemia

Category of public health significance	Prevalence of anemia (%)
Severe	≥ 40
Moderate	20.0 – 39.9
Mild	5.0 – 19.9
Expected	≤ 4.9

2.5.8 Iron deficiency (ID)

Transferrin receptor levels >8.0 µg/l for children 6-59 months old and non pregnant women of child bearing age were used to indicate iron deficiency (WHO 2001).

2.5.9 Iron deficiency Anemia (IDA)

An individual was classified with IDA if s/he was simultaneously categorized with both iron deficiency (high TfR) and anemia (low haemoglobin) according to the criteria outlined above.

2.5.10 Vitamin A deficiency (VAD)

Vitamin A deficiency was defined as RBP less than 0.70µmol/l (Sommer 2002). The presence of infection and/or inflammation was assessed using C-reactive protein (CRP) and Alpha acid-1 glycoprotein (AGP). An elevated CRP concentration (>5 mg/L) and an elevated AGP concentration (>1.2 mg/L) indicates the presence of an acute phase response due to infection and/or inflammation (Erdhardt 2006). The prevalence of vitamin A deficiency was calculated in two ways, 1) not excluding for inflammation (ie elevated acute phase proteins) and 2) excluding the vitaminA results of women and children with inflammation identified by a CRP >5mg/L and/or AGP >1.2 mg/l.

Sub-clinical vitamin A deficiency is defined as retinol binding protein < 0.7 µmol/L, while severe vitamin A deficiency is defined as retinol binding protein < 0.35 µmol/L. The public health burden of vitamin A deficiency can be determined based on the prevalence of low serum retinol in preschool children, as proposed by the WHO (Table 2.13). In addition, criteria proposed by the International Vitamin A Consultative Group (IVACG) state that a prevalence of serum retinol concentration < 0.7 µmol/L >15% among children 2-5 years of age constitutes a public health problem.

Table 2.13 Public health burden of vitamin A deficiency based on serum retinol ≤0.70µmol/L in children ≥ 1 year (WHO 1996)

Public health burden of vitamin A deficiency	Prevalence of low serum retinol (%)
Mild	≥2 to ≤10
Moderate	>10% to <20
Severe	≥20

2.5.11 C- reactive protein (CRP) analysis

The acute phase proteins (APP) are biomarkers for sub-clinical inflammation. The APP includes C-reactive protein (CRP) and Alpha-1-acid-glycoprotein (AGP). CRP is an indicator of acute inflammation, because plasma concentration of CRP starts to increases 6 hours after on-set of infection, it reaches maximum 24 to 48 hours later and start decreasing thereafter. AGP is an indicator of chronic inflammation, because plasma concentration of AGP is slow to rise and reaches maximum about 2 to 5 days after infection (Biesalski 2007).

The prevalence of infection acute phase proteins was assessed by the elevation of C-reactive protein (CRP). According to the manufacturer, a concentration of >5 mg/L indicated an acute phase response and subjects with positive CRP test results were excluded from analysis of vitamin A deficiency.

2.5.12 Alpha 1-acid glycoprotein (AGP) Analysis

The prevalence of chronic infection acute phase proteins was assessed by the elevation of Alpha 1-acid glycoprotein (AGP). According to the manufacturer, a concentration of >1.2mg/L indicates an acute phase response. The vitamin A data of all individuals with elevated AGP (>1.2mg/L) were excluded from the data used to indicate prevalence of vitamin A deficiency.