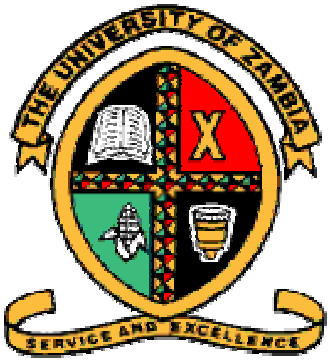
Report of the National Survey to Evaluate the Impact of Vitamin A Interventions in Zambia In July and November 2003


THE NATIONAL
FOOD AND NUTRITION
COMMISSION OF ZAMBIA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ACKNOWLEDGMENTS

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The survey report was prepared by Christine Clewes and Chipepo Kankasa, and statistical analyses were performed by Jean Welsh and James Campbell.

These findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency.

EXECUTIVE SUMMARY

This report summarises the findings of the Zambian National Vitamin A Survey conducted in households throughout Zambia in July and November 2003. The collaborating partners include the United States Agency for International Development (USAID), the Micronutrient Operational Strategies and Technologies (MOST) Washington and MOST Zambia, the University Teaching Hospital, Zambia, the National Food and Nutrition Commission (NFNC) and the International Micronutrient Malnutrition Prevention and Control (IMMPaCt) Program team from the Centers for Disease Control and Prevention (CDC)Atlanta. Funding was provided by the CDC through an inter-agency agreement with USAID.

SURVEY OBJECTIVES

The overall objective of the assessment was to evaluate the impact of vitamin A interventions in Zambia, namely sugar fortification with vitamin A and the supplementation of post-partum women and the twice yearly supplementation of children aged 6 to 59 months with vitamin A capsules. The specific objectives were as follows:

- To determine the prevalence of vitamin A deficiency (VAD) in children (6–59 months) and non-pregnant women of child-bearing age (15–49 years)
- To compare the prevalence of VAD shortly after vitamin A supplementation at a Child Health Week (CHW) to the prevalence 4 months later
- To compare the prevalence of VAD to that found in the National Survey on VAD in Zambia in 1997
- To determine the prevalence of anaemia in children (6–59 months) and non-pregnant women of child-bearing age (15–49 years)
- To estimate the coverage of vitamin A supplementation and fortified sugar
- To compare two methods for the analysis of vitamin A levels in sugar
- To determine the prevalence of households using iodized salt (July only).

SURVEY METHODS

Survey design and sample size

A sample of 390 households was selected in July and November from 30 non-stratified clusters that were previously randomly selected for use in the Demographic and Health Survey (DHS) in Zambia (2001). These clusters were used because current detailed maps of them were available and no further enumeration was needed. The same clusters, but not necessarily the same households, were used in the first and second phases of the survey, which facilitated planning and community mobilisation and reduced the standard error when comparing the results from the first and second phases. In each cluster, 13 children aged 6 to 59 months and their mother or female caregiver, a non-pregnant woman of child-bearing age (15–49 years), was randomly chosen to participate in the study. If a caregiver was a man, a pregnant woman, or an older woman, only the child-related data were collected. A large enough sample of children was selected to allow a comparison of results between the data collected in July and that collected in November.

Consent

The parent or guardian of the selected child was asked for his or her consent and for consent for the child to take part in the survey (Appendix B)

Questionnaire

Each caregiver completed a pre-tested standardised questionnaire, which had been translated into 7 major Zambian languages, and which was read to the respondent by an interviewer in the language normally used in the household (Appendix I). The questionnaire covered questions on demographics, morbidity, attendance and knowledge of CHW, child feeding history, knowledge of the sugar fortification programme, and purchase and use of fortified sugar. All participating households received a 1- kg bag of fortified sugar on completion of the interview.

Blood sample

In addition to the completed questionnaire, a finger-prick sample of blood was taken from the child and the caregiver, if the person was a non-pregnant woman aged 15 to 49 years. Where the caregiver was a pregnant woman, a woman outside of the age range, or a man, no blood sample was taken and only questions concerning the child were completed.

The first drop of blood was used to make a thick smear malaria slide; the rest of the blood (up to 500 μL) was collected into a microtainer containing the anti-coagulant, lithium heparin. The haemoglobin concentration was tested at the household with blood from the microtainer and measured using the HemaCue™ system. The remaining blood sample in the microtainer was kept cold and in the dark, until it could be centrifuged later in the day and the plasma removed. The plasma was frozen and later analysed for retinol (vitamin A) and the indicators of infection: C-reactive protein (CRP), and α-1-acid glycoprotein (AGP).

Other Tests

When available, a small sugar sample was taken from the household, and a bag of sugar was purchased from the nearest shop to the cluster for vitamin A analysis. In July only, salt samples from the household were tested for the presence of iodine.

SUMMARY OF FINDINGS

Vitamin A Deficiency (VAD)

Children

The overall mean plasma retinol concentration of children 6 to 59 months was 0.71μmol/L (standard deviation [SD] 0.25) which was better than that found in the last vitamin A survey conducted in 1997, when the mean plasma retinol concentration was 0.64μmol/L (National Food and Nutrition Commission 1997 [NFNC]). In the 1997 survey, the prevalence of VAD in children, defined as retinol concentrations ≤ 0.7 μmol/L was 65.7%, indicating a severe public health problem (WHO 1996). In July 2003, 53.3% (95% confidence intervals [CI]: 44.3, 62.4) of children had VAD and in November 54.7% (95% CI: 45.3, 64.1) had VAD. The overall percentage of VAD in children for the two surveys was 54.1% (95% CI: 46.5, 61.6). The result showed improvement from 1997, but still represented a severe public health problem in Zambia. The prevalence of severe VAD, defined as a plasma retinol concentration less than 0.35 μmol/L (WHO 1996) was 5.0%, less than half of that in 1997 (11.7%).

The prevalence of VAD in children was higher in the presence of elevated CRP and AGP concentrations, malaria parasites in the blood, anaemia, reported illness in the past 2 weeks and lower level of education of the caregiver. Having received a vitamin A capsule during the last CHW, residing in a household where fortified sugar was available, being given anthelmintics, the

demographics of the family, or the mother having received a post-partum supplement was not associated with the prevalence of VAD in children.

A recent meta-analysis of the effect of sub-clinical infection, as measured by acute phase proteins, on plasma retinol concentrations and prevalence of VAD showed that it was possible to correct plasma retinol values for the presence of an acute phase response (Thurnham *et al*, 2003). After correction, the retinol concentration of the combined July and November data for children who had an elevated CRP concentration increased from 0.62µmol/L to 0.77µmol/L. The overall plasma retinol concentration of children with normal CRP concentrations was 0.82µmol/L.

Women

The overall mean plasma retinol concentration for women aged 15 to 49 years was 1.13 (SD 0.41) µmol/L. Using the WHO cut-off for VAD, a retinol concentration ≤0.7 µmol/L, the prevalence of VAD was 13.4% (95% CI: 9.4, 17.4), reflecting a substantial improvement from the 1997 prevalence of 21.5% (NFNC, 1997). The prevalence of VAD was higher among women with recent fever, elevated CRP and anaemia. It was not significantly different however, by reported cough or diarrhoea, in households where fortified sugar was available, or in women with elevated AGP concentrations. Vitamin A status was better in women who used oestrogen-based products for family planning.

VITAMIN A SUPPLEMENTATION

Children

Approximately 95% of women said they had heard of the CHW, and about 91% attended the June CHW with their children. Overall, 87% of mothers reported that their children had received a vitamin A supplement in June however, fewer 66% reported that their children had received a supplement in the January CHW.

Women

Post-partum vitamin A supplements were reported to have been taken by 39.0% (95% CI: 30.9, 47.0) of women compared with 21% in 1997 (NFNC, 1997). The prevalence of VAD in women receiving vitamin A supplements at any time in the last 3 years was not significantly different from those who had not received supplements. The number of women who had received a supplement in the last 6 months was too small to analyse statistically.

Although reported use of post-partum vitamin A supplements had no relationship with VAD, it was associated with a reduced prevalence of anaemia.

FORTIFIED SUGAR

Fortified sugar consumption

Approximately 59% of household respondents reported buying fortified sugar on a regular basis, whereas only 46% identified sugar as a food fortified with vitamin A. Cost appeared to be a constraining factor on how much sugar was bought, as 55% of households used more sugar at the beginning of the month than in the middle or end of the month. Seasonality also had an effect, as overall, 38% of caregivers reported they consumed less sugar during the rainy season (November to May). "Whitespoon" sugar, produced by Zambia Sugar, was purchased by 57% of households in the 30 days up to the day of the survey; 32% of households bought 25-g repackaged bags of sugar of unknown origin.

Children consumed sugar mostly in porridge (39%), in tea (31%), or in cereal (12%). Among women who consumed sugar, 38% reported using it with tea, 17% with porridge and 14% with cereal. Other foods with sugar were eaten by less than 2% of the population.

Quality of vitamin A fortified sugar

About 82% of sugar samples collected from the households were inadequately fortified, which is below the minimum legal level for fortification of <10 mg of vitamin A per kg of sugar. The consistency of sugar fortification levels across provinces indicated that the distance the sugar was transported did not have an effect on the level of fortification nor did the type of sugar purchased.

Comparison of the results from 2 methods of estimating retinol in sugar

The two methods for assessing vitamin A in sugar, spectrophotometry (the gold standard) and fluoremetry, gave good agreement at lower concentrations of vitamin A, but above 15 mg retinol equivalents (RE) of vitamin A per g of sugar, the fluorescence assay gave significantly lower values. The reason for the difference is that the calibration curve for the fluoremeter tended to level off at higher concentrations. To use the fluorescence method, it is necessary to dilute sugar samples of high concentration to get them into the range where the method is accurate.

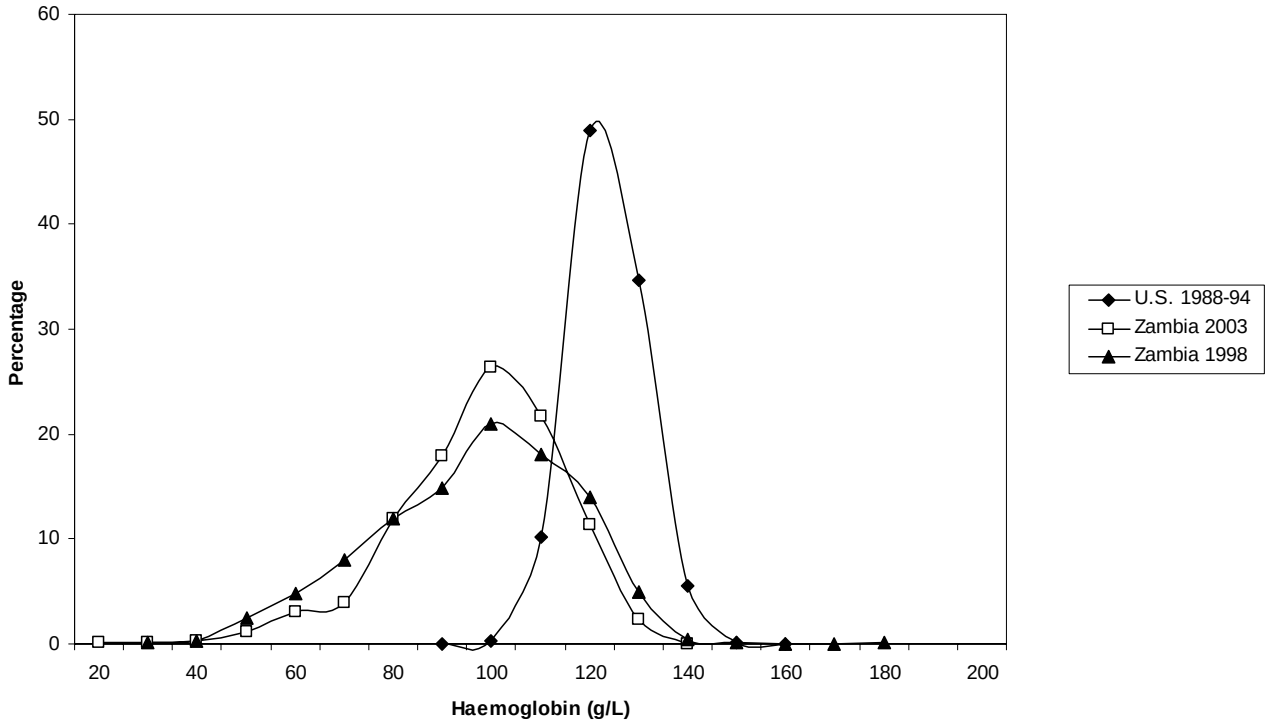
ANAEMIA

Children

Overall, 52.9% (95% CI: 46.7, 59.3) of children had haemoglobin concentrations <less than110 g/L in 2003, an improvement compared with the last survey in 1998 when 65% (95% CI: 62, 67) of children were anaemic (NFNC 1999). In July, 59.3% (95% CI: 51.5, 67.0) of children surveyed were anaemic with haemoglobin concentrations less than 110g/L. A small percentage (3%) was severely anaemic, with a haemoglobin concentration between 40 and 70 g/L. In November, the situation was slightly better as 47.0% (95% CI: 39.2, 54.8) of children were anaemic, and only 1% were severely anaemic. Anaemia in Zambian children is still a severe public health problem, based on WHO standards (WHO 2001).

The distribution curve of haemoglobin concentrations in children 6 to 59 months in 2003 showed some improvement compared with data collected in Zambia in 1998 (Figure A). It was, however, still skewed to the left and had a wider distribution compared with data collected in the US National Health and Nutrition Examination Survey (NHANES) III (1988–94) for children 12 to 48 months (Cogswell, unpublished data)**.**

Figure A: Haemoglobin distribution of Zambian children 6-59 months and American children 12-48 months of age



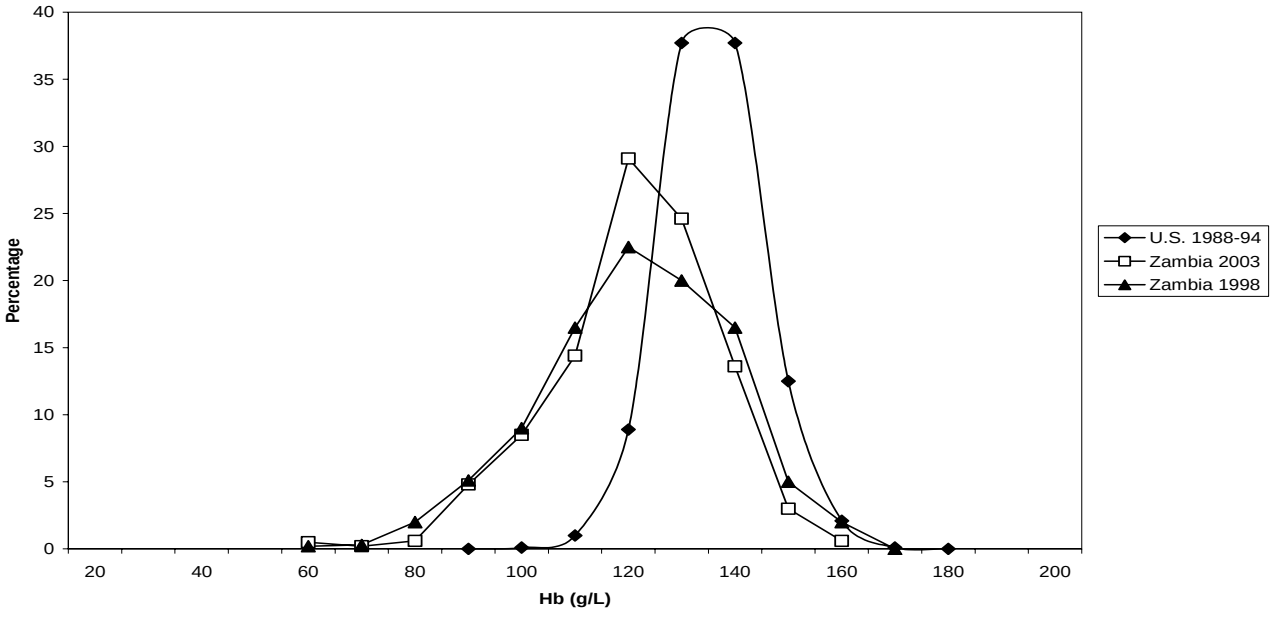
The prevalence of anaemia was higher among those with elevated CRP concentrations, malaria parasites in the blood, VAD, and reported diarrhoea or fever. The prevalence of anaemia did not significantly differ by reported cough, living in a household where fortified sugar was available, vitamin A supplementation, the demographics of the household, or the education of the mother. Children given anthelmintics at the June CHW had a lower prevalence of anaemia than those who were not dewormed.

Non-pregnant women

The mean haemoglobin concentration in non-pregnant women aged 15 to 49 years was 126 (SD 15) g/L. Anaemia, defined as a haemoglobin concentration < 120 g/L, was found in 29.1% (95% CI: 24.7, 33.4) of women, hence, anaemia is a moderate public health problem (WHO 2001). Very few women (0.3 %) were severely anaemic.

The distribution curve for haemoglobin concentrations of Zambian women aged 5 to 49 years is broader and more skewed to the left compared with that of US women aged 20 to 59 years from the NHANES III data (1988–94) (Cogswell, unpublished data) but showed improvement compared with the Zambian data collected in 1998.

Figure B: Haemoglobin distribution of Zambian women 15-49 years and American women 20-59 years



The prevalence of anaemia did not vary significantly by elevated concentrations of CRP, the presence of malaria parasites in the blood, consumption of fortified sugar, reported morbidity, night-blindness, the education or demographics of the mother. The prevalence of anaemia was lower among women using oestrogen-based family planning methods and women reporting having received post-partum vitamin A supplements. Conversely, women who had VAD (retinol ≤ 0.7μmol/L) were significantly more likely to have anaemia than women with a normal vitamin A status.

SIGNIFICANCE OF FINDINGS

Children

The results of the survey have shown that VAD is still a serious public health problem in children 6 to 59 months of age in Zambia as 54% of children have plasma retinol concentrations ≤ 0.7 μmol/L. Part of the apparent failure of the children to respond to the vitamin A supplementation programme may be attributable to the high levels of sub-clinical infection present in the population, and asymptomatic malaria may be having the biggest effect. This does not mean however, that giving vitamin A capsules has not had any beneficial effects. The plasma retinol concentrations of children with no infection were significantly higher (0.82μmol/L) than those with infection (0.62μmol/L). The retinol in those children with infection at the time of the survey may have been used by the tissues or may have been stored in the liver, but measuring plasma retinol only provides information on the circulating levels. The survey highlights an apparent limitation of using circulating plasma retinol as a measure of vitamin A status in populations. Plasma retinol measured by HPLC is currently the gold standard until alternative methods can be verified.

Anaemia was found in 53% of children 6 to 9 months, although this is an improvement on the data reported in 1998, when 65% of children were anaemic (NFNC, 1999), anaemia is still a severe public health problem (WHO 2001). The prevalence of anaemia appears to parallel that of VAD perhaps because both are increased in the presence of infection and VAD has been shown to be associated with anaemia (Fisherman et al 2000; WHO/UNICEF 2004).

Improving the nutritional status of young children through multiple approaches (e.g. good breast-feeding practices, high coverage of vitamin A supplementation, adequate fortification of sugar, fortifying other foods in addition to sugar, increasing the diversity of the diet and encouraging home

gardening) may reduce the impact of malaria morbidity and reduce susceptibility to other diseases, and should be considered within a package of interventions. For example, to reduce the disease burden of malaria, the distribution of treated bed-nets was introduced as part of the CHW strategy in December 2003. The widespread use of bed-nets, especially for women and their pre-school children may help reduce the number of mosquito bites they may be exposed to, which in turn could lead to a reduction in symptomatic and asymptomatic malaria. As a consequence, there would be a reduction in the number of acute phase reactions, which would allow plasma retinol and haemoglobin concentrations to increase, hence improving the overall vitamin A and haematological status of the population.

Women

In non-pregnant women 15 to 49 years there has been a significant shift towards reducing VAD as the percentage of women with retinol concentrations ≤ 0.7 μmol/L has been reduced from 21.5% in 1997 to 13% in 2003. The presence of infection was negatively associated with vitamin A status and the recommendations for intervention in the section above are true for women as well.

Use of post-partum vitamin A supplements in the last 3 years did not have any effect on VAD. The vitamin A supplement is probably most effective during the first 6 months post-partum, but there were not enough women in the sample size to test the short-term effectiveness. It is important, however, to remember the beneficial effect of post-partum vitamin A supplements to women and their newborns and to try to encourage women to ask for their post-partum supplement or to try to incorporate it into other routine clinic attendances (e.g. when women bring infants for BCG vaccination within 6 weeks of birth).

The breast-feeding practices of 70% of women did not comply with current WHO recommendations to exclusively breast-feed for the first 6 months of the infant's life, as many were introducing complementary foods too early. Exclusive breast-feeding for the first 6 months of the infant's life should be promoted for women who are HIV negative or do not know their status (WHO 2003); women who are HIV positive should be given the facts to help them make an informed decision (WHO/UNICEF 2004).

Use of oestrogen-based family planning practices was associated with lower prevalence of both VAD and anaemia.

Sugar

Fortification of sugar with vitamin A has been shown to be a highly effective strategy for reducing VAD in other countries and is an important public health strategy in Zambia. The survey results highlight the need to strengthen the enforcement and monitoring of the regulations to ensure the sugar is fortified at an adequate level.

FUTURE

At a round table discussion held in Lusaka on 9 September 2004, the results of the 2003 surveys were discussed. Recommendations for the future are summarised below. In addition, it was proposed that an intensive study "Absorption and retention, and longer-term impact of high-dose vitamin A supplements on total body vitamin A stores of 3– 4 year old Zambian children" be undertaken.

RECOMMENDATIONS

Vitamin A

Child health weeks (CHW)

To strengthen the implementation of CHWs at community and district level, there is need to:-

- Encourage community innovation,
- Encourage community participation to take ownership,
- Encourage private health facilities to take part,
- Improve coverage of vitamin A supplementation in all districts,
- Use advocacy to improve programme support from policy makers.

Child–feeding practices should:-

- Promote exclusive breast feeding in the first 6 months for women who are HIV negative or who do not know their status. For HIV positive women, when replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all breastfeeding is recommended. Otherwise, exclusive breast feeding is recommended during the first months of life and should be discontinued as soon as it is feasible.

- Thereafter infants and young children should receive safe and nutritionally adequate complementary foods with continued breastfeeding up to 2 years of age, where appropriate (see above).
- Explore low dose (50,000 IU) early supplementation, as a way of ensuring an adequate supply of vitamin A to infants during the first six months. The proposed early vitamin A supplementation can be linked to routine immunization.

Women

Improve the postpartum vitamin A capsule supplementation programme by:-

- Linking supplementation to BCG immunisation of children within 6 to 8 weeks of delivery
- Distribution of the vitamin A in maternity wards

Food fortification

- Fortification of sugar with vitamin A is an important strategy to improve the vitamin A status of the Zambian population, and it is essential to strengthen enforcement and monitoring of the regulation of the statutory instrument (No.155) to ensure adequate and consistent vitamin A fortification. The statutory instrument states that packaged sugar be sold with a minimum fortification level of 10 mg per kg sugar.
- Explore vitamin A fortification of other commonly consumed foods (e.g. maize meal, cooking oil and milk).

Dietary diversification

- Consumption of vitamin A–rich foods is one of the long-term strategies that can improve vitamin A status, therefore, the promotion, production and consumption of vitamin A–rich foods, such as red palm-oil and yellow fleshed sweet potato, should be encouraged.

Infection

- Distribution of treated bed-nets should continue as part of the CHW programme. Heavy disease burden, especially through malarial parasites, depresses circulating concentrations of retinol.

- Deworming should continue as part of CHW.
- Other public health interventions should be encouraged (e.g. clean water, sanitation).
- To reduce exposure to infection and improve both vitamin A and iron status, education about reducing exposure to infection should be promoted (e.g. use of bednets, hand washing etc.).

Iron

To strengthen the programme to reduce anaemia the following recommendations were proposed:

- Targeted interventions to provide iron supplements to the most vulnerable population groups (e.g. children < 5 years of age and women, especially pregnant women),
- Food-based approaches to increase iron intake through food fortification (e.g. flour). Continuing to include a deworming programme as part of CHW, as the use of anthelmintics was associated with a lower prevalence of anaemia in children,
- Continue to include the distribution of treated bed-nets as part of the CHW programme. Use of bednets will reduce the number of mosquito bites, which will reduce sub-clinical and clinical malaria, resulting in a reduction in the prevalence of anaemia,
- Education to increase awareness and knowledge among health care providers and the general public on the health risks associated with anaemia,
- Dietary diversification

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ACT	α-1- anti-chymotrypsin
AGP	α-1-acid glycoprotein
CDC	Centers for Disease Control and Prevention
CHW	Child Health Week
CI	Confidence intervals
CRP	C-reactive protein
CSAs	Census Supervisory Areas
DHMB	District Health Management Board
DHS	Demographic Health Survey
FDLC	Food and Drug Control Laboratory, Zambia
FRAT	Fortification Rapid Assessment Tool
GLM	General linear model
Hb	Haemoglobin
HH	Household
HPLC	High pressure liquid chromatography
IMMPaCt	International Micronutrient Malnutrition Prevention and Control Program
IVACG	International Vitamin A Consultative Group
KAP	Knowledge, Attitude, Practice questionnaire
MoH	Ministry of Health
MOST	Micronutrient Operational Strategies and Technologies
NFNC	National Food and Nutrition Commission, Zambia
SD	Standard deviation
SEAs	Standard Enumeration Areas
UNICEF	United Nations Children's Fund
UNZA	University of Zambia
USAID	United States Agency for International Development
VAD	Vitamin A deficiency
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

Background

Zambia is situated in southern Africa and takes its name from the Zambezi River, which rises in the northwest corner of the country and forms its southern boundary. The landlocked country lies between latitudes 10^{o} and 18^{o} south and longitudes 22^{o} and 33^{o} east. It is bordered by the Democratic Republic of Congo to the north and northwest, Tanzania to the northeast, Malawi to the east, Mozambique to the southeast, Zimbabwe to the south, Botswana and Namibia to the southwest and Angola to the west (see map).

Figure 1: **Map of Zambia**

Zambia has one of the lowest population-to-land ratios in Africa with only 10 million people in a country half the size of Western Europe (752,612 square kilometers). The country is divided into 9 provinces: Central, Copperbelt, Eastern, Luapula, Lusaka, Northern, North-Western, Southern

and Western. The employment opportunities offered in the post-independence era (1964) in the copper mines and associated industries led to a strong rural-to-urban migration. The result is that Zambia has become one of the most urbanised countries in Africa with about one fifth of the population living in the Copperbelt. The largest concentration of people however, is in Lusaka with an estimated population of over 2 million. There are 7 main languages spoken: Bemba, Kaonda, Lozi, Lunda, Luvale, Nyanja and Tonga; English is also spoken as an official language.

1.2 Climate and Crops

Zambia has a tropical climate and vegetation with 3 distinct seasons:

1. A warm, wet season from November to April.
2. A cool, dry season from May to August.
3. A hot, dry season during September and October.

The climate in Zambia is suitable for the cultivation of a wide range of crops: maize, tobacco, cotton, rice, wheat, groundnuts, tea, coffee and sugar cane. It is also possible to grow a variety of vegetables and fruits including citrus fruit, bananas, pineapples, mangoes, and avocados. However, recent droughts in 1992–1993, 1994–1995 and 2002–2003 agricultural seasons have caused losses in maize production in 5 of the 9 provinces. Normally carry-over stocks and imports make up shortfalls, but the scarcity of foreign exchange has made it difficult to buy imported grain, which has threatened food security.

1.3 Health and Nutrition

The total fertility rate for Zambia is 5.7 children per woman, with an estimated birth rate of 38.9 births per 1000 population, and an infant mortality rate of 98.4 deaths per 1000 live births (WHO, 2004). More than half of the children under 5 years of age in Zambia are stunted, with the highest levels recorded in the provinces of Luapula (63%), Eastern province (64%) and the Southern province (44%) (UNICEF, 2004). The levels of child malnutrition in Zambia showed improvement throughout the 1990s, but since 1999 have deteriorated quite significantly, which has been attributed to a combination of drought and the impact of HIV/AIDS. Because of the drought, children in northern Zambia did not have access to adequate supplies of maize, their staple diet, while districts in southern Zambia with a better road network had access to the maize available in bigger markets. Despite the better road networks, the more urbanised areas showed a greater degree of deterioration in child malnutrition, perhaps because these areas have become more vulnerable to HIV/AIDS (UNICEF, 2004).

1.4 HIV/AIDS

AIDS is a major public health challenge in Zambia. The UNAIDS report on the Global HIV/AIDS Epidemic (2002) estimates that 21.5% of Zambians between the ages of 15 and 49 years were infected with HIV at the end of 2001, with the rate rising to 25% among young rural women and 31% among women in urban antenatal care clinics. As a result, some new vulnerable groups have emerged in recent years, including households with AIDS orphans, those with very high dependencies due to chronic sickness, households that have lost productive adults due to HIV deaths, migrant and single-parent households (UNICEF, 2004).

It is estimated that about 10 to15% of children in Zambia who are less than 5 years of age may have HIV/AIDS. About 25,000 infants, about a third of Zambian infants born to HIV-infected women are infected with HIV each year *in utero*, during delivery, or through breast feeding. Exclusive breast-feeding protects infants against severe diarrhoea and acute respiratory illness, which are other major causes of infant death, and evidence from South Africa suggests that exclusive breast feeding may protect infants against HIV transmission (Coutsoudis, 2005). Mixed feeding (breast milk plus infant formula or other breast milk substitutes) may be the most dangerous practice in areas of high HIV prevalence (Coutsoudis *et al,* 2001), and unfortunately, although the majority of Zambian infants are breast fed, only 26% of infants 0 to 3 months and 5% of infants 4 to 5 months of age are exclusively breast fed (Linkages, AED 2004).

1.5 Vitamin A situation in Zambia

Vitamin A deficiency (VAD) has been known to be a problem in Zambia for some time and the Government of Zambia is addressing the problem principally through vitamin A supplementation of post-partum women and children 6 to 59 months, fortification of sugar, education and dietary improvement. Supplementation of children is accomplished during Child Health Weeks (CHW). Sugar fortification, which began in 1998, and is mandated by law, is viewed as an appropriate longer-term approach to improving the vitamin A status of the population.

1.5.1 Historical Efforts to combat VAD using supplements

Efforts to combat VAD in Zambia began in 1990 with a commitment by the government to provide vitamin A supplements to vulnerable populations. By 1992, vitamin A capsules were being distributed to children 6 to 72 months of age and to lactating mothers in drought-affected areas. The distribution was then extended to health centres throughout the country targeting the

same groups. In 1996, the Zambia Demographic Health Survey indicated that VAD prevalence might be high, as infant mortality rates (108/1000) and under-5 mortality rates (192/1000) were high. A baseline national vitamin A survey was conducted in 1997, funded by the United States Agency for International Development (USAID), which reported that 66% of Zambian children had serum retinol concentrations ≤ 0.7 μmol/L (National Food and Nutrition Commission, [NFNC], 1997). The survey found only 28.4% of children younger than 5 years and 13.5% of postpartum mothers had received vitamin A supplements. The conclusion that was reached based on the 1997 survey was that a supplementation coverage rate of at least 65% was necessary to reduce mild xerophthalmia by 75 to 80% in children younger than 4 years.

1.5.2 Food fortification in Zambia

The fortification of margarine with vitamin A began in Zambia in 1978, but because consumption was low, especially among the poorer groups of the population, it had little impact on national vitamin A status. Between 1992 and 1995 the NFNC and the Ministry of Health (MoH) examined the implications of food fortification as a complementary effort to supplementation. The initial food identified for fortification was maize, as it is the daily staple, but rising costs for maize meal and the hundreds of hammer mills used to mill the maize were major deterrents to using this food. Attention then shifted to sugar as a possible vehicle. It was thought that at least half the population consumed sugar, and it was expected that consumption would increase. In addition, sugar was used in children's food, especially porridge, and children were an important population group to target.

The minimum vitamin A fortification level for sugar was based on the daily average consumption of 15 g/day by children. The maximum fortification level was calculated based on three times the average adult daily sugar consumption, 48 g/day. It was thought that half the initial retinol content would remain in the sugar over its shelf life. Using these data, it was calculated that sugar should be fortified with vitamin A so that at the retail level, the concentration would be 15 mg/kg sugar, equivalent to 30% of the Recommended Dietary Allowance (RDA). The sugar fortification programme began in May 1998, but cost considerations led the sugar company to reduce the fortificant level to 10 mg/kg sugar within 3 months of the start of the programme.

The Zambian Statutory Instrument No. 155 was enacted on December 18, 1998 and stated that packaged sugar should be sold with a minimum fortification level of 10 mg vitamin A per kg sugar, the legal minimum level.

1.5.3 Evaluation of the supplementation and fortification programmes

Currently, there is no external quality control (QC) of fortified sugar at production or retail levels. The Food and Drug Control Laboratory (FDCL) has not been able to analyse sugar samples because of lack of funds for transport and reagents. Fortification of sugar is expected to reach the whole population, with poorer families consuming less, but there are limited data available, and very little data on intra-household use of sugar.

Data from mini-surveys in selected areas of Zambia show that the vitamin A supplementation programme for children achieved over 70% coverage for the February 2000 CHW and over 80% coverage for two successive rounds in August 2000 (combined approach for distribution using sub-National Immunization Days [NIDs] and CHW) and February 2001. It was expected that, given the high coverage rate it may be possible to measure a reduction in VAD prevalence.

Government policy based on World Health Organization (WHO) recommendations state that post-partum women should be given a 200,000 IU vitamin A supplement within 6 weeks post-partum. Although, supplement coverage is high for children 6 to 59 months, coverage for post-partum women is expected to be much lower.

1.6 Justification for vitamin A impact assessment

The Zambian government and USAID were interested in determining the impact of the vitamin A supplementation and fortification programmes they support. In addition, the data obtained from Zambia could contribute to the international evidence about vitamin A programmes. It was hoped that there had been an overall improvement in vitamin A status in the population and that this could be documented. To obtain the data, a survey planning team composed of the International Micronutrient Malnutrition Prevention and Control Program (IMMPaCt) at the Centers for Disease Control and Prevention (CDC), the Micronutrient Operational Strategies and Technologies (MOST) Washington, MOST Zambia and USAID Zambia developed a plan for an impact assessment of vitamin A programmes in Zambia. The CDC provided the funding, via an inter-agency agreement with USAID, and also provided technical assistance for the micronutrient survey.

1.6.1 Justification for measuring haemoglobin

It has long been known that VAD and iron deficiency tend to exist together in population groups because diets are usually deficient in more than one nutrient (Sight and Life 2001). The prevalence of anaemia associated with VAD has not been estimated, but is thought to be widespread especially in women and young children. The mechanism of vitamin A–related anaemia is unclear, but it has been shown that VAD restricts the release of iron from the iron stores (Sommer and West, 1996). Controlled trials in several countries have shown vitamin A supplementation was associated with a significant increase in haemoglobin concentration, with best results obtained when vitamin A was in amounts close to recommended levels of intake (Sommer and West, 1996). Because of the interaction between vitamin A and iron, it was thought that haemoglobin concentration would give an indication of anaemia status in children 6 to 59 months and non-pregnant women 15 to 49 years of age following the introduction of the vitamin A intervention programmes in Zambia.

1.7 Survey objectives of assessment

The overall objective of the survey was to evaluate the impact of vitamin A interventions, namely the post-partum supplementation of women and the twice yearly supplementation of children 6 to 59 months of age with vitamin A capsules and sugar fortification in Zambia.

The specific objectives were as follows:

- To determine the prevalence of VAD in children (6–59 months) and non-pregnant women of child-bearing age (15–49 years),
- To determine the change in prevalence of VAD shortly after a CHW and 4 months later,
- To compare the prevalence of VAD to that found in the1997 vitamin A survey,
- To estimate the household coverage of vitamin A supplementation and fortified sugar,
- To determine the prevalence of anaemia in children (6–59 months) and non-pregnant women of child-bearing age (15–49 years),
- To compare two methods for the analysis of vitamin A in sugar,
- To determine the prevalence of households using iodized salt (July only).

CHAPTER 2: METHODOLOGY

2.1 Ethical clearance

The survey protocol was approved by an Ethics Committee in Zambia and was determined to be public health practice through the Research Determination procedure at CDC.

2.2 Survey design

It was determined that simply mimicking the baseline survey, which was conducted just before fortification began in 1997, would not provide enough information on the impact of supplementation as well as fortification. It was, therefore, decided to use a 2-phased national study design:

- A national random sample survey approximately 1 month following the vitamin A supplementation of children through CHW. This would be a best-case scenario reflecting the full impact of supplementation in addition to sugar fortification.
- A national random sample survey at the end of the supplementation cycle, just before the next round of vitamin A capsule distribution. This would provide information on the longer-term effectiveness of the supplementation programme.

In theory, the survey just after supplementation would capture the specific impact of the national supplementation programme, whereas the end point survey would give an idea of the decline in vitamin A status over the post-supplementation period, and also allow for the assessment of the residual impact potentially attributable to sugar fortification, i.e. the effect of the vitamin A supplement would be minimal in November and any remaining improvement in status compared to 1997 may be due to the sugar fortification programme.

2.3 Population groups

- Young children (6–59 months of age)
- Non-pregnant women of child-bearing age (15–49 years)

Young children 6 to 59 months were included in the survey because they are the targeted group for vitamin A supplementation through CHW in Zambia. The rationale for surveying non-pregnant women was to include a population whose vitamin A status would be minimally affected by supplementation, but who may have had some impact from fortification. Some women may

have received post-partum supplements, but this number was expected to be low (< 20%). In the survey in 1997, the reported vitamin A supplementation in women was low (i.e. 14% of women received vitamin A capsules) (NFNC, 1997). Thus, it was expected that any improvement in the vitamin A status of non-pregnant women may be due to sugar fortification.

2.4 Sample size

Children
The 1997 survey found an overall prevalence of 66% VAD, defined as serum retinol ≤ 0.7µmol/L in children 6 to 59 months. The following sample size calculations were used for each of the two surveys carried out in 2003:

We estimated the prevalence of VAD shortly after supplementation to be about 30% and at the end of the cycle to be about 45%. To differentiate between these prevalences, at a precision of ± 8% and an α risk of 5%, and a design effect assumed at 2 in both surveys, a minimum of 253 and 298 children were needed.

Women
The 1997 survey found that 21.5% of women had serum retinol concentrations of ≤ 0.7µmol/L. Based on that data, the following calculations were made:

We estimated that the prevalence of VAD may have been reduced to 15% due to the sugar fortification programme. In order to achieve a precision of ± 4% at an α risk of 5%, and a design effect assumed to be 2, a sample size of 613 woman was required.

To account for > 25% of refusals or "dropouts" (for example, no blood samples obtained, child's caregiver is currently pregnant, etc) the total number of women required was increased to 383 for each survey, a total of 766 women. Finally, to simplify the data collection procedures, the number of women and children to be recruited was increased to 390 for both the July and November surveys. The data collected from the women in both 2003 surveys was combined to ensure a large enough sample size for statistical analysis.

2.6 Sampling design

As both interventions (vitamin A supplementation and sugar fortification) are nationally implemented programmes, only a single national survey sample was used, and stratifying the

sample across geographical areas was not warranted. One consideration was to stratify the sample by programme coverage rates, but coverage information on either supplementation or fortification was not strong enough to support stratification. The proposed sample design was, therefore, a non-stratified national cluster survey. The Demographic and Health Survey (DHS) in Zambia completed data collection in 2001. Therefore, 30 clusters were randomly selected from those used by the DHS (permission obtained from the Statistics Office). One of the main reasons for utilizing the DHS clusters was that current detailed maps of those clusters were available and no further enumeration was needed. The same clusters, but not necessarily the same households were used for both surveys in July and November 2003. The reasons were:

- The same maps could be used for both surveys,
- the survey teams would be familiar with the areas after the first survey,
- The chosen communities would be sensitized to both surveys,
- There would be a smaller standard error when comparing the data from the two time periods.

The survey included 30 clusters with 13 children and women in each. One randomly chosen child, aged 6 to 59 months and his or her mother or caregiver were selected per household. The selection was done by asking a responsible adult how many children there were in the household under the age of 5 years. If there was more than one child in the eligible age group, then each child was listed by age and a random table was used to randomly select one of the eligible children (see questionnaire, appendix I). Following selection of the child, the adult was asked if he or she was the regular caregiver for that child and, if so, then he or she was also chosen. If the normal caregiver was not present, but was due to return within a reasonable length of time, then two further attempts were made to recruit the caregiver. If on the third attempt the person had not returned to the household, the household was not included in the survey and the team went to the next household.

Informed consent was obtained from each caregiver. Where the caregiver was a woman, consent was obtained from her husband if she requested his consent, before proceeding with the survey (see appendix B for the informed consent forms).

2.7 Indicators

2.7.1 Knowledge, Attitude, Practice (KAP) Questionnaire

The KAP questionnaire included questions from the mini surveys conducted in Zambia after each CHW, from the DHS 2001 survey and from the Fortification Rapid Assessment Tool (FRAT) (PATH, 1998). The questionnaire was drafted in English and pre-tested, modified and translated into the seven main languages of Zambia. Each language was then back translated into English to ensure an exact translation. The questionnaire covered the following:

- Family demographic information;
- Information about the child, such as age, cough, diarrhoea and fever during the previous 2 weeks, whether the child had been dewormed in the last 6 months, breast and complementary feeding history, attendance at CHW;
- Information about the non-pregnant woman (15–49 years) such as education, age, pregnancy status, family planning practices, as well as morbidity questions on diarrhoea, cough, fever and night blindness;
- Vitamin A supplementation coverage information included recall data from the last supplementation cycle for young children and the last post-partum dose of vitamin A taken by the mother/caregiver if a non-pregnant woman 15 to 49 years of age;
- Questions on night blindness during last pregnancy — to distinguish between night blindness and general eye sight problems, a question on day vision was also asked;
- Use of oestrogen-based birth control methods of the non-pregnant woman because of the known positive effect of the hormone on retinol concentrations;
- Knowledge about vitamin A-fortified foods;
- Sugar consumption of young children and women of child-bearing age.

See attached questionnaire in appendix I

2.7.2 Biological samples

Capillary blood samples were taken from both the non-pregnant woman and her child. If the caregiver was pregnant, male or not of eligible age for the survey, only a sample from the child was collected. A trained nurse or laboratory technician with each field team collected capillary blood by pricking the second or third finger with a Tenderlett lancet (International Technidyne Corporation, Edison, USA). The first drop of blood was used for a thick smear slide for malaria assessment (Appendix E). Then up to 500 µl of whole blood was collected into a microtainer (Becton Dickinson, USA) containing lithium heparin as an anti-coagulant. After mixing with the

anti-coagulant, a drop of blood was drawn into a HemoCue™ cuvette and tested for haemoglobin in a HemoCue™ photometer (HemoCue™ AB, Sweden). Referral notices to the local health centre were given to those subjects found to be anaemic. The remaining whole blood in the microtainer was kept cool in an insulated box with cold blocks until the end of each survey day when it was processed.

2.7.3 Sample type

Either a serum or plasma retinol sample with a concentration of $\leq 0.7\mu mol/L$ can be used to identify VAD in children (Sommer 1995). To obtain the maximum amount of sample for analysis, especially in an area where there is a high prevalence of infection in the population, we chose to collect plasma. Where there are high infection rates, there are likely to be high concentrations of fibrinogen in the blood. The presence of fibrinogen can make it difficult to obtain the maximum amount of serum from a clotted blood sample. The use of an anti-coagulant followed by centrifugation to produce plasma can also be affected by high concentrations of fibrinogen, but a larger volume of sample is more likely using this method.

2.7.4 Biological sample processing and storage

At the end of each field day, the laboratory technicians, with the help of other team members, either used the portable centrifuge they were carrying to spin the whole blood or took the blood samples to the nearest hospital or health clinic that had a centrifuge. If the team had a base from which they worked each day, they transferred the plasma into cryovials and stored them in a freezer until the end of the field work. If the team did not return to the same place each night, the plasma was stored in a refrigerator overnight and kept on ice in cold boxes during the following days. The team covering the Northern and Luapula clusters had samples collected over the first 5 days transported to Lusaka by a specially hired vehicle; they then continued on for the second half of their field work. At the end of the field study, plasma samples from all clusters were transported on ice packs to a freezer at University Teaching Hospital (UTH), Zambia.

2.7.6 Food samples

Sugar

Household sugar samples (50–100 g) were collected where available and stored in small black plastic bags. A bag of sugar was purchased from the nearest shop to each cluster and stored in a similar way.

Salt

Household salt samples were tested at the household during the July round of the survey only. The rapid test kit (MBI kits, Madras, India) was used to test for the presence of iodine. The results were categorised as no colour change, meaning it was unlikely the salt contained any iodine, or a colour change, meaning the salt was likely to contain some iodine but the amount was not quantified.

A 1 kg bag of fortified sugar was given to all households participating in the survey.

A summary of all the methods of assessment and indicators used in the surveys can be found in Table 1.

Table 1: Summary of survey components.

Methods of assessment and indicators	**Children 6 – 59 months**	**Women of childbearing age, 15 – 49 years**	**Household samples**
VITAMIN A			
Plasma vitamin A by High Pressure Liquid Chromatography (HPLC)	x	x	
Household sugar samples			x
MEASURES OF INFECTION			
α-1-Acid glycoprotein (AGP)	x	x	
C-reactive protein (CRP)	x	x	
ANAEMIA			
Haemoglobin by HemoCue	x	x	
Thick smears for malaria	x	x	
IODINE			
Salt samples-test kit (July only)			x
QUESTIONNAIRE	x	x	

2.8 Logistics

2.8.1 Survey Teams

Personnel were selected by MOST Zambia, NFNC and the principal survey co-ordinator in Zambia. Laboratory technicians, nurses and interviewers with previous survey experience were sought from throughout Zambia to ensure all languages required were covered. The final composition of each of the six field teams was: one supervisor, one interviewer, one nurse, one laboratory technician and one driver.

2.8.2 Training

All team members (except the driver) received instruction regarding the overall survey objectives and procedures. Interviewers responsible for administering the survey questionnaire participated in role-playing interviews to ensure consistency. Pre-testing of the questionnaire was also conducted during the training. The nurses and laboratory technicians were trained on blood collection and processing techniques. The supervisors were familiar with all techniques but refresher training was given.

Two pilot surveys, one in an urban and one in a rural district, were carried out in the Lusaka area before the first phase of the survey. One pilot survey was carried out before the November survey. All pilot surveys were carried out in clusters not selected for the main surveys.

2.8.3 Fieldwork

The first survey was conducted during the cool, dry season, 21st to the 31st July 2003 and followed the most recent national vitamin A supplementation cycle in the middle of June. The second survey took place at the end of the hot, dry season and was in the first week of November (3rd to the 8th November) just before the next round of vitamin A supplementation at the beginning of December.

2.8.4. Community Mobilization

It was expected that the local District Health Management Boards (DHMB) would be notified in advance of the survey in July, but this had not been done in some areas, resulting in some time delays at the start of the survey. In the Copperbelt region, the survey team was refused entry to two clusters: an army camp and a mining area. The director of NFNC secured permission from the army HQ in Lusaka for the survey, but the paperwork did not reach the local army camp in

time. The cluster had to be replaced. The director of the DHMB in Chingola tried to get the mine owner to agree to the survey but was not successful, this cluster also had to be replaced.

2.9 Laboratory specimens

2.9.1 Laboratory analysis

The malaria thick smears were analyzed by the Tropical Disease Research Centre (TDRC) in Ndola, Zambia (see method in Appendix E).

A sample volume of 150 µl of plasma was required for the following assays: plasma retinol, C-reactive protein (CRP) and α-1-acid glycoprotein (AGP). Plasma retinol concentrations were measured by high pressure liquid chromatography (HPLC) at the Stellenbosch laboratories in South Africa. The retinol assay was done in duplicate. Where possible, two biochemical measures of infection, the acute phase proteins, CRP and AGP, were analysed by nephelometry to correct plasma retinol concentrations for the presence of infection. Methodologies for all measurements are provided in appendix E.

Prior to sample analysis, the CDC laboratory sent known plasma retinol samples to the South African laboratory to help ensure the quality of the methods used at Stellenbosch. CDC also provided control samples to ensure quality control for both the acute phase proteins and retinol assays. Additional high and low controls for CRP and AGP were purchased from the manufacturers of the reagents for the acute phase protein analyses.

Sugar samples were analyzed at FDCL in Zambia using spectrophotometry (Arroyave and Funes, 1974) and the CRAFTi flurometric method developed by Craft Technologies (Craft et al, 2004).

CHAPTER 3: DATA ANALYSIS

3.1 Data entry

During the field survey, questionnaires were checked by the supervisors at the households, or if that was not possible, before leaving the cluster. Any errors in completing questionnaires were corrected by going back to the households to verify answers, if necessary. All questionnaires were double entered into a database using EpiInfo 6.4. To reduce computer data entry errors, the entry screen was programmed to accept only codes within a predetermined range. Following data entry, the data were cleaned and analysed using SPSS version 11.0 and SAS version 9.0 at CDC.

3.2 Definitions of respondents

"Caregiver" — At each household the usual caregiver for the selected child aged 6 to 59 months was asked to take part in the survey. In the majority of households the caregiver was the mother of the child who was a non-pregnant woman and between the ages of 15 and 49 years. In some households, the caregiver was a pregnant woman, the grandmother, or the father or other male caregiver. The caregiver gave informed consent, completed the family demographic information, the child information and the sugar consumption data.

"Woman" — when the caregiver was a non-pregnant woman between 15 and 49 years, the woman completed the section on women's information in the questionnaire and gave a blood sample, in addition to the information collected as described under "caregiver".

3.3 Assessment of vitamin A prevalence

In 1997 estimates of the prevalence of VAD in children 6 to 59 months were provided by two different cut-offs, plasma retinol < 0.35 μmol/L and ≤ 0.7 μmol/L, and for ease of comparison the same cut-offs were used in 2003 (WHO 1996). A plasma retinol value of ≤ 0.7 μmol/L was indicative of the presence of sub-clinical vitamin A deficiency; a concentration < 0.35 μmol/L indicates severe VAD. No assessment for clinical signs were made. In addition, the WHO proposed the following guidance: if ≥ 2 – < 10% of children have serum retinol values ≤ 0.7 μmol/L, the population has a mild vitamin A public health problem, ≥10 – < 20% is considered a moderate public health problem and ≥ 20% a severe public health problem (WHO 1996).

To estimate the prevalence of low plasma retinol in women of childbearing age (15 – 49 years) a cut-off of < 1.05 μmol/L is used (Pilch, 1987). In the 1997 survey, however, a cut-off for plasma retinol of ≤ 0.7μmol/L was used, so this report will also use this cut-off to be able to make a direct comparison with the earlier data.

To distinguish between night blindness, caused by low plasma retinol during pregnancy, and general eye sight problems in women who had a live birth in the last 3 years, the prevalence of daytime vision problems and the prevalence of night-time vision problems with no daytime problems, was calculated. A prevalence of >5% of women with night blindness indicates VAD is a significant problem in the population (Sommer and Davidson, 2002).

3.4 Assessment of anaemia prevalence

Assessment of true iron status requires the measurement of a number of parameters. As the main focus of the survey was to assess the impact of measures to combat VAD, only haemoglobin was used as a measurement of anaemia. Low haemoglobin indicates anaemia and two cut-offs were used to estimate the prevalence of anaemia in children 6–59 months: haemoglobin concentrations < 70 g/L and < 110 g/L (WHO 2001). The age-related criteria for normal haemoglobin were developed by CDC from NHANES II data (Expert Scientific Working Group, 1985). Based on a cut-off of 110 g/L, an anaemia prevalence of 5 to 19.9% represents mild public health problem, whereas 20% to 39.9% represents a moderate problem and ≥ 40% a severe problem in a population (WHO, 2001). A cut-off of 70 g/L indicates severe anaemia (WHO, 2001).

To estimate the prevalence of anaemia in non-pregnant women of childbearing age (15–49 years) two haemoglobin cut-offs were used < 70 g/L and < 120 g/L. Based on the cut-off of 120 g/L, the same definition of a public health problem as stated above applies.

3.5 Assessment of prevalence of infection (acute phase proteins)

CRP is a fast acting acute phase protein and a concentration > 5 mg/L indicates the presence of an acute phase response (Gabay 1999). AGP is a slower acting acute phase protein usually elevated during chronic infection, and an arbitrary cut-off of >1 g/L is used. In the presence of an elevated acute phase protein, plasma retinol concentrations can be depressed, hence, the use of plasma retinol cut-offs alone can overestimate VAD. To overcome this problem, where CRP alone is elevated, plasma retinol concentrations were adjusted by multiplying the plasma retinol values by

1.25; where AGP alone was elevated, the plasma retinol was multiplied by a factor of 1.18 (Thurnham *et al,* 2003). There were too few samples analysed to correct for both CRP and AGP being elevated simultaneously.

3.6 Assessment of malaria prevalence

Almost all of the malaria cases (95%) in Zambia are caused by *Plasmodium falciparum* and although malaria is endemic, the worst season is during the rainy season from November to May. Malaria rates have been gradually increasing in Zambia since 1976, and in 1999 there were 3.5 million cases (Africa Fighting Malaria, 2002). As is found in other places, the incidence of malaria among children under the age of 5 years is the highest of all population groups. In 1999, the estimated national incidence of malaria among children under 5 years in Zambia (980 per 1000) was almost six times that of the incidence among children over 5 years (169 per 1000) and nearly 40% of the deaths of children aged 5 years or under are caused by the disease. The worst hit province was the Northwest province, followed by the Western and Southern provinces (Africa Fighting Malaria, 2002). Although the numbers quoted are based on clinical malaria, asymptomatic malaria can have an adverse impact on retinol concentrations. Therefore, plasma retinol data were analysed in children and women with and without malaria parasitaemia.

3.7 Data cleaning and weighting

A database was compiled to track all samples (e.g. sugar, plasma, and slides) collected. Ages of some of the respondents were not known or were out of the specified age range in the target groups. In those instances when the calculated ages of the children were < 6 months or > 60 months and those of the women were < 15 or > 49 years, the subjects were removed from the data set. Where haemoglobin concentrations were below 40 g/L or plasma retinol concentrations were < 0.1 μmol/L, these values were excluded from the data sets.

SAS 9.0 was used for data analysis to account for the cluster sampling methodology used when calculating the measures of precision (confidence intervals [CI] and standard deviations [SD] or standard errors [SE]). All data related to the children were weighted to adjust for the total number of children aged 6 to 59 months in the selected household.

3.8 Sample size

A total of 385 households in July and 389 households in November were surveyed in the 9 provinces throughout Zambia. After data cleaning, 380 household questionnaires in July and 386 in November were available to analyse. Table 3.1 shows the sample sizes for the questionnaire, malaria slides, haemoglobin, plasma retinol and acute phase protein data for children 6 – 59 months.

Table 3.1: Sample Size for data collected for children aged 6 – 59 months.

	July	November	Combined
Questionnaire	380	386	766
Malaria	367	373	740
Haemoglobin	353	371	724
Plasma retinol	317	342	659
C-Reactive Protein (CRP)	119	178	297
α-1-acid glycoprotein (AGP)	17	50	67

The sample sizes for the questionnaire, malaria slides, haemoglobin, plasma retinol and acute phase protein data for the non-pregnant women of child-bearing age (15 – 49 years) are provided in Table 3.2. Data obtained in July and November were combined to have enough statistical power for analysis. (See section 2.4).

Table 3.2: Sample Size for mothers/female caregivers aged 15 – 49 years.

	July	November	Combined
Questionnaire[1]	361	379	740
Haemoglobin[2]	299	324	623
Malaria[2]	298	324	622
Retinol[2]	283	300	583
CRP[2]	112	144	256
AGP[2]	16	49	65

[1] All women [2] Non-pregnant women

CHAPTER 4: DEMOGRAPHICS

4.1 Demographic characteristics of the households

It is widely known that the demographic characteristics of a population can have an influence on nutritional status. A better understanding of these characteristics, particularly education, is useful to help understand the distribution of nutrition indicators in a population.

4.2 Head of household and principal language spoken

The caregiver who was interviewed was asked whether the head of the household was male or female and the principal language spoken by the family. Among those interviewed, the majority said the head of the household was male (88%) and the most common language spoken in the home was Bemba (Table 4.1).

Table 4.1: Sex of head of household and main language spoken in the home

Head of Household	July % (n = 379)	November % (n = 385)	Combined % (n = 764)
Male	87.6	84.7	86.1
Female	12.4	15.3	13.9
Main Language	(n = 374)	(n = 384)	(n =758)
Bemba	41.4	39.6	40.5
Nyanja	17.4	21.1	19.3
Lunda	0.8	0.5	0.7
Luvale	4.8	5.2	5.0
Kaonde	3.7	3.4	3.6
Lozi	5.9	5.2	5.5
Tonga	12.8	13.8	13.3
Others	13.1	11.2	12.1

4.3 Formal education women

Having received a formal education is associated with the ability to read and write and with better understanding, which could influence the individual's decision making on nutrition and health

care issues. Mothers/female caregivers were, therefore, asked the highest level of education attained. Of the 739 women asked, 9% had received no formal education. Only 31% had completed primary school education, although 60% had attended (Table 4.2). Just over one-quarter of the women were educated to secondary school level or higher.

Table 4.2: Highest level of education attained by mother/female caregiver

Highest Level Attained	July and November Combined data % (n = 739)
No formal education	9.1
Primary school not completed	29.2
Primary school completed	30.9
Secondary school	28.8
Higher education	2.0

4.4 Age Distribution of Sample

4.4.1 Children

Children 6 to 59 months were eligible for the survey. Only 16% of children were in the 6 to 11.9 month age group because of the narrower age range (Table 4.3). The low percentage of children in the 48 to 59.9 month age group cannot be explained.

Table 4.3: Age grouping of children surveyed.

* Age Groups (months)	July % (n = 380)	November % (n = 386)	Combined % (n = 766)
6 – 11.9	16.1	15.3	15.7
12 – 23.9	23.5	31.1	27.3
24 – 35.9	19.5	18.4	19.0
36 – 47.9	25.6	22.0	23.8
48 – 59.9	15.3	13.2	14.2

* Weighted analysis

4.4.2 Women

Out of the 766 caregivers interviewed, 740 were non-pregnant women between 15 and 49 years of age. The majority (57%) were between 20 and 30 years of age (Table 4.4) and the mean age of the women was 28.4 years.

Table 4.4: Age distribution of the non-pregnant women.

Age group years	July and November Combined data % (n = 740)
15 – 19.9	5.4
20 – 29.9	56.6
30 – 39.9	30.3
40 - 49.0	7.7

CHAPTER 5: MORBIDITY

5.1 Reported Morbidity

This chapter provides information on biochemical and reported morbidity data for the women and children surveyed. As part of the child information section in the questionnaire, each caregiver were asked whether their child had suffered from fever, cough, or diarrhoea during the 2 weeks prior to and including the day of the survey. Each caregiver was also asked if their child had been treated for worms in the last 6 months. Women who were of eligible age were asked similar questions about their own health, that is, if they had suffered from diarrhoea, cough or fever in the last 2 weeks. In addition, to assess the prevalence of night blindness as an indicator of VAD, they were asked about their ability to see during the day and at night during their last pregnancy.

5.1.1 Reported morbidity in children 6 to 59 months of age.

Overall, 46.6% of children were reported to have had a fever, 63.9% to have suffered from a cough and 32.2% to have experienced a bout of diarrhoea in the 2 weeks prior to the survey (Table 5.1). In July, 82.3% of children were reported to have had an illness in the 2 weeks prior to the interview. In November, the percentage was slightly lower, as 74.1% of children were reported to have suffered from cough, fever and/or diarrhoea, giving an overall percentage of 78.2% of children who were reported to have been ill within 2 weeks of each of the surveys.

Table 5.1: Prevalence of fever, cough or diarrhoea in the 2 weeks prior to the survey, and treatment with anthelmintics in the last 6 months, reported for children aged 6 – 59 months.

Child morbidity	July		November		Combined	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Fever	377	50.8 (42.4, 59.1)	386	42.5 (37.0, 47.9)	763	46.6 (41.0, 52.2)
Cough	380	69.1 (63.3, 74.8)	386	58.7 (53.2, 64.2)	766	63.9 (60.1, 67.6)
Diarrhoea	378	29.7 (22.9, 36.5)	385	34.7 (28.1, 41.3)	763	32.2 (26.8, 37.6)
Any illness	380	82.3 (77.5, 87.1)	386	74.1 (69.6, 78.6)	766	78.2 (74.8, 81.5)
Dewormed	375	62.6 (54.8, 70.4)	378	48.8 (39.9, 57.6)	753	55.7 (48.0, 63.3)

One of the interventions of the CHW is to treat children with anthelmintics to remove intestinal worms such as *Ascaris lumbricoides*. Each caregiver was asked if their child had been dewormed in the last 6 months. In July, 63% and in November, 49%, reported that their children had been dewormed in the last 6 months (Table 5.1).

5.1.2 Morbidity in women 15 – 49 years of age.

Overall, there was not as much infection reported for women compared with their children.

Table 5.2: Self-reported fever, cough or diarrhoea among women of child-bearing age in the 2 weeks prior to the survey.

Women morbidity	July and November Combined data	
	n	% (95% CI)
Fever	735	28.4 (22.2, 34.6)
Cough	738	41.1 (37.2, 44.9)
Diarrhoea	733	10.4 (7.2, 13.5)

5.1.3 Night blindness in women

Women who fulfilled the eligibility criteria were asked if, when they were pregnant with their last child, they had difficulty seeing during the day. They were then asked the same question about their vision at night.

Table 5.3: Difficulty seeing during the last pregnancy

	July and November Combined data	
	n	% (95% CI)
Difficulty seeing during the day	523	19.9 (13.1, 26.7)
Difficulty seeing at night but not in the day	527	2.3 (0.8, 3.7)

Analysis was limited to women's most recent pregnancy that resulted in a live birth during the 36 months up to the month of the survey. Based on the calculations described in section 3.2, the prevalence of pregnancy-related night blindness in women was 2.3%.

5.2 Biochemical measures of morbidity

Due to a lack of adequate plasma volume after duplicate analyses of retinol, measurements of CRP and AGP were only possible on a limited number of samples. CRP analysis was carried out on 256 non-pregnant women and 297 child samples. AGP analysis was done on 65 women and 67 child samples.

5.2.1 Malaria and acute phase proteins in children

In July, 40.1% of thick smear blood slides were positive for malaria parasites (Table 5.4). In November, the percentage was lower, and only 21.4% were positive, giving an overall result of 30.7% of children with malaria-positive slides.

Expressed as a percentage of the total children surveyed, 39% of samples were analysed for CRP and 9% for AGP. Because there were so few AGP test results, only the combined data for July and November are presented. Of the samples analysed, approximately 40% (95% CI: 31.5, 47.4) of children had an elevated CRP and 76% (95% CI: 58.7, 93.5) had an elevated AGP.

Because of the small number of samples analysed for the acute phase proteins, the demographics of children for whom there was enough plasma to do an acute phase protein analysis were compared with those who did not have enough plasma, to ensure there were no differences. There were no significant differences between those with and those without a CRP measurement.

Table 5.4: **Prevalence of abnormal acute phase proteins C-reactive protein (CRP) > 5 mg/L and α-1-acid glycoprotein (AGP) > 1 g/L and malaria-positive slides in children 6 – 59 months of age.**

Biomarker	July		November		Combined Data	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Malaria positive	367	40.1 (27.9, 52.3)	373	21.4 (12.3, 30.6)	740	30.7 (20.6, 40.8)
CRP >5 (mg/L)	119	45.4 (34.1, 56.7)	178	35.6 (26.4, 44.7)	297	39.4 (31.5, 47.4)
AGP >1 (g/L)		--		--	67	76.1 (58.7, 93.5)

5.2.3 Malaria and acute phase proteins in women

The overall percentage of malaria-positive slides was 7.3 % (Table 5.2), considerably lower than the 30.7% of positive slides for children.

A smaller percentage of women had acute phase protein concentrations outside of the normal range compared with children. Elevated CRP concentrations were measured in 22.7% (95% CI: 17.0, 28.3) and elevated AGP concentrations in 23.1% (95% CI: 12.5, 33.7) of samples.

Table 5.5: Prevalence of abnormal acute phase proteins, C-reactive protein (CRP) ≥ 5 mg/L and α-1-acid glycoprotein (AGP) ≥ 1 g/L and malaria-positive slides in non-pregnant women 15 – 49 years of age.

Biomarker	n	July and November (Combined data) % (95% CI)
Malaria positive	622	7.3 (4.1, 10.3)
CRP >5mg/L	256	22.7 (17.0, 28.3)
AGP > 1g/L	65	23.1 (12.5, 33.7)

CHAPTER 6: CHILD FEEDING HISTORY

WHO (2003) recommends that on a population basis, exclusive breastfeeding for the first 6 months is the optimal way of feeding infants. Thereafter, infants and children should receive complementary foods with continued breastfeeding up to 2 years of age or beyond.

Table 6.1: Age at which child given complementary foods

Age (months) given complementary food	July* % (n = 355)
1	1.9
2	4.8
3	15.7
4	21.5
5	12.1
6	36.1
>6	7.9

*Weighted analysis

The majority of mothers, 69.7% (95% CI: 64.9, 76.9), gave complementary foods between 4 and 6 months of age, whereas 22.4% gave weaning foods before 4 months and 7.9% reported that they had given complementary foods late, that is, after the age of 6 months (Table 6.1). There was no usable variable for the introduction of complementary foods from the November survey so no data are presented.

CHAPTER 7: VITAMIN A SUPPLEMENTATION

7.1 Vitamin A supplementation of children aged 6 to 59 months

Questions on vitamin A supplementation and on the Child Health Weeks (CHW) were asked to understand the caregiver's awareness of the CHW and to assess how many caregivers took their children to the twice yearly event. Questions particularly targeted the supplementation of vitamin A to children 6 – 59 months of age, which is an important part of the CHW.

7.2 Familiarity of the caregiver with CHW

The majority of caregivers (94.7%) said they had heard of the CHW, and 88.9% attended the June CHW with their children (Table 7.1).

Table 7.1: Familiarity with Child Health Weeks (CHW)

	July		November		Combined	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Heard of CHW	376	94.1 (91.1, 97.2)	383	95.3 (92.4, 98.2)	759	94.7 (92.6, 96.8)
Took child to June CHW	357	91.6 (89.6, 94.6)	373	86.3 (82.8, 89.0)	730	88.9 (86.2, 91.6)

Caregiver's knowledge of CHW

The question, "Please describe what happened when you took your child to the CHW", was designed to confirm the caregiver had attended the CHW by asking her or him to remember, without prompting, events that happened while they were there with their children.

Approximately, 80% of the caregivers remembered the vitamin A capsule, even if they did not remember the capsule being cut to release the drops. In addition, two-thirds of caregivers remembered their children receiving immunisation (Table 7.2).

Table 7.2: Number of CHW activities remembered by caregiver who took child to a CHW

More than one answer allowed for this question.

Description of Activities	July (n = 380) %	November (n = 386) %	Combined (n = 766) %
Vitamin A capsule mentioned	81.3	80.6	80.9
Capsule cut	21.1	17.4	19.2
Distribution site mentioned	14.5	15.3	14.9
Growth monitoring & promotion	12.1	19.7	15.8
Immunization received	63.7	61.9	62.8
Health education received	4.7	13.0	8.9
Other intervention received	14.5	17.6	16.1

7.4 Child received vitamin A supplements at the CHW in January and June

The percentage of caregivers who remembered their child receiving vitamin A in June and/or January was consistent across the two surveys; therefore, suggesting that the caregiver did accurately remember attending or not attending the CHW. Fewer children received vitamin A in January (65.5%) than June (87.3%).

Table 7.3 Percentage of children who received vitamin A during the January and June CHW

Child received vitamin A	July		November		Combined	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
CHW January	354	64.7 (55.9, 73.4)	360	66.4 (58.8, 73.9)	714	65.5 (58.6, 72.4)
CHW June	376	89.8 (85.9, 93.7)	376	84.8 (80.5, 89.0)	752	87.3 (83.8, 90.8)

*Weighted analysis

7.5 Child received doses of vitamin A at other times since January 2003

In general, vitamin A supplements given during CHWs were not recorded on health cards. When a child was given vitamin A at health clinics or hospitals, however, it was recorded in the child's health card. The question, "Has the child received any vitamin A supplements since January", was designed to get some idea of how many children were getting vitamin A supplements at time points other than CHWs. The information given by the caregiver was confirmed by checking the child's health card.

Table 7.4: Percentage of children who received vitamin A supplements (not part of CHW) since January.

	July		November		Combined	
	n	%	n	%	n	%
Child received vitamin A since January	**356**	**22.2**	**354**	**25.2**	**710**	**23.7**

About a quarter of children were reported to have received a vitamin A capsule at a time other than a CHW since January 2003 (Table 7.4).

7.6 Post-partum vitamin A supplementation of women

Zambian government policy based on WHO recommendations state that post-partum women should be given a 200,000 IU vitamin A supplement within 6 to 8 weeks of the birth of their children. Among the 527 women who delivered their last child in the past 36 months, 39.0% (95% CI: 30.9, 47.0) reported that they received vitamin A after delivery (not shown).

CHAPTER 8: PREGNANCY STATUS AND BIRTH CONTROL PRACTICES OF THE WOMEN

8.1 Number of women pregnant at time of survey

The women of childbearing age were asked whether or not they were pregnant. Of the 740 mothers/female caregivers interviewed, 731 responded to the question regarding current pregnancy status. Of these, 91 (12.4%) reported that they were pregnant.

8.2 Type of family planning used by the women

Among non-pregnant women, it was important to know what proportion were using oestrogen based birth control methods because of the positive effect of the hormone on retinol concentrations. Approximately 31% of women reported they were using oestrogen-based methods. The remaining women were either using no birth control or other non-oestrogen based products (Table 8.1).

Table 8.1 Birth control methods practiced.

Birth control method used	Combined data (n = 643) %
Birth control pills, norplant	31.3
No birth control	68.3
Other birth control	0.5

CHAPTER 9: SUGAR CONSUMPTION AND KNOWLEDGE OF VITAMIN A

9.1 Commercial foods containing vitamin A in Zambia

At the time of data collection, sugar and margarine — see section 1.5.2 — were the only foods fortified with vitamin A in Zambia. The final part of the questionnaire was designed to assess the percentage of caregivers who knew about the benefits of vitamin A fortified sugar, what percent bought fortified sugar, and if they did not, what were the major constraints that prevented the families from buying sugar.

Table 9.1: Knowledge of foods fortified with vitamin A.

Food Fortified with Vitamin A	Combined data (n = 764) %
Sugar	45.9
Oil	0.7
Other	4.7
No foods are fortified	0.3
Don't Know	48.4

The combined results from questionnaires in July and November showed that less than half of the caregivers, 45.9% (95% CI: 35.8, 54.1) knew that sugar was fortified with vitamin A, and 48.4% didn't know if any foods were fortified (Table 9.1).

9.2 Purchase of fortified sugar

Table 9.2: Purchase of fortified sugar.

Purchase of fortified sugar	Combined data (n= 728) %
Fortified sugar normally purchased	58.5
Fortified sugar is not normally purchased	8.5
Don't know	33.0

Fortified sugar was reportedly purchased by 58.5% (95% CI: 49.8, 67.1) of caregivers on a regular basis, substantially more than the 46% of caregivers who knew that sugar was fortified

(Table 9.2). The amount of sugar purchased at the two time points was fairly consistent, as in July 56.4% (95% CI: 46.2, 66.6) and in November 60.4% (95% CI: 51.6, 69.1) of households reported that they purchased fortified sugar (not shown).

9.3 Cost of fortified sugar

To assess whether money might be a constraining factor on how much sugar was bought, the caregiver was asked if the rate of sugar consumption was constant throughout the month. Nearly half said the amount of sugar they bought from month to month did not vary (Table 9.3).

Table 9.3 Household Monthly Sugar Consumption

Amount of sugar bought during month	Combined data (n = 761) %
Monthly sugar consumption constant	46.9
Monthly sugar consumption not constant	44.3
Don't know	8.8

9.4 Consumption pattern of sugar

When specifically asked (in July only) if the household used more sugar at the beginning of the month compared with the middle or end of the month, the majority of caregivers (54.6%) said they had more sugar at the beginning of the month. Approximately a quarter of caregivers said they did not know whether the amount of sugar they bought varied during the month (results not shown).

9.5 Seasonal pattern of sugar consumption

In rural areas in particular, the availability of money to buy sugar might be seasonal. Therefore, a question was asked about seasonal variation in purchasing sugar. In the July survey, approximately half of the households (47%) said they consumed less sugar during the rainy season compared with the dry season. However when the same question was asked in the November survey, just before the start of the rainy season, only 29% of caregivers said they consumed less sugar in the rainy season. A quarter thought they consumed more (results not shown).

9.6 Twenty-four hour recall of foods eaten with sugar

An effort was made to estimate what sugar-containing foods had been eaten by women and their children in the previous 24-hour period. Sugar was most often consumed by children as tea with sugar (31%) or porridge with sugar (39%), whereas a small percentage ate cereal with sugar (12%) (Table 9.4a). The women also drank tea with sugar (38%), but fewer ate porridge with sugar (17%). Approximately the same percentage of women as children ate cereal with sugar (14%) (Table 9.4b). Questions were asked about other foods eaten with sugar in Zambia, for example, beans or bean soup, sour milk, scones, fritters, potatoes, or traditional brew. In general, less than 2% of women and children ate any of these or other foods with sugar.

Table 9.4a: Foods with sugar eaten by children aged 6–59 months old: 24-hour recall.

Foods with sugar eaten by children	July		November		Combined	
	n	%	n	%	n	%
Tea	371	33.5	385	28.7	756	31.0
Porridge	364	37.8	386	40.5	750	39.2
Cereal	349	9.5	385	13.9	734	11.8

Table 9.4b: Foods with sugar eaten by women of child-bearing age: 24-hour recall

Foods with sugar eaten by women	Combined	
	n	%
Tea	730	38.1
Porridge	716	17.2
Cereal	715	14.1

9.7 Sugar availability in the household

Women were asked about the brand of sugar they had purchased in the last 30 days and 57% stated that they had bought Whitespoon sugar (produced by Zambia Sugar). Because of the cost of sugar, many local shop-keepers re-package sugar into small unlabelled plastic bags containing approximately 25g of sugar per bag. Overall, about one-third of households bought this type of re-packaged sugar (Table 9.5).

Table 9.5: Type of sugar purchased by respondents during previous month prior to survey.

	July (n = 323) %	November (n = 318) %	Combined (n = 641) %
Whitespoon sugar	59.8	54.4	57.1
Kalungwishi sugar	0.0	3.8	1.9
Imported sugar	1.9	3.5	2.7
Unknown brand-repackaged	35.3	28.9	32.1
Unknown brand-loose from open sack	0.6	0.6	0.6
Unknown brand original package	1.5	0.9	1.2
Other	0.9	7.9	3.7

A sample of sugar was collected from households that had sugar and agreed to give a sample for analysis. A few households (6.2%) refused to give a sugar sample and some (50.5%) had none available on the day of the survey. A total of 326 sugar samples were collected for analysis (Table 9.6). In November, fewer households had sugar available than in July, 59.6% versus 40.9%.

Table 9.6: Percent of households from which sugar was collected.

	July n =367 %	November n = 386 %	Combined N = 753 %
Sugar sample collected	50.1	36.8	43.3
No sugar collected -refused	9.0	3.8	6.2
No sugar available in household	40.9	59.6	50.5

9.8 Adequacy of sugar fortification

Sugar samples were analysed spectrophotometrically at the Food and Drug Control Laboratory (FDCL) in Lusaka, Zambia. The Statutory Instrument in Zambia (1998) states that packaged sugar must be sold with a minimum fortification level of 10 mg vitamin A per kg sugar. This assumes that sugar will be fortified at a higher level at the factory, as there will be some fortificant degradation by the time the sugar has reached the point of sale. Only 17.6% (95% CI: 12.3, 22.9) of the 301 household sugar samples were at or above the minimum legal level, whereas 37.2%

(95% CI: 31.1, 43.3) were inadequately fortified with vitamin A (2.5 – <10 mg/kg), and 45.2% (95% CI: 38.8, 51.6) had < 2.5mg/kg of vitamin A. (Figure 9.1).

Figure 9.1*:* **Adequacy of sugar fortification.**

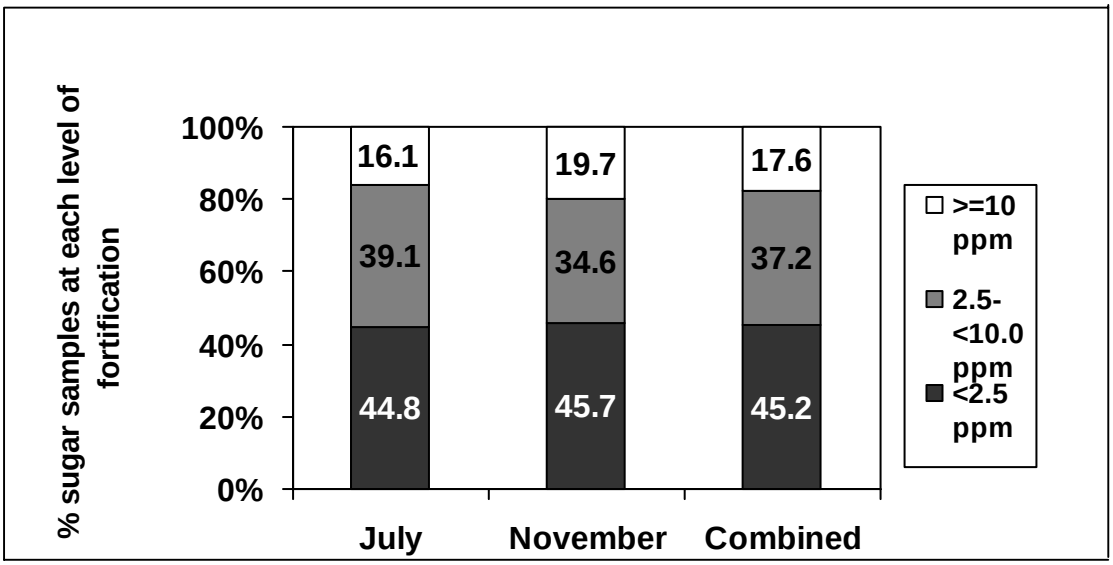
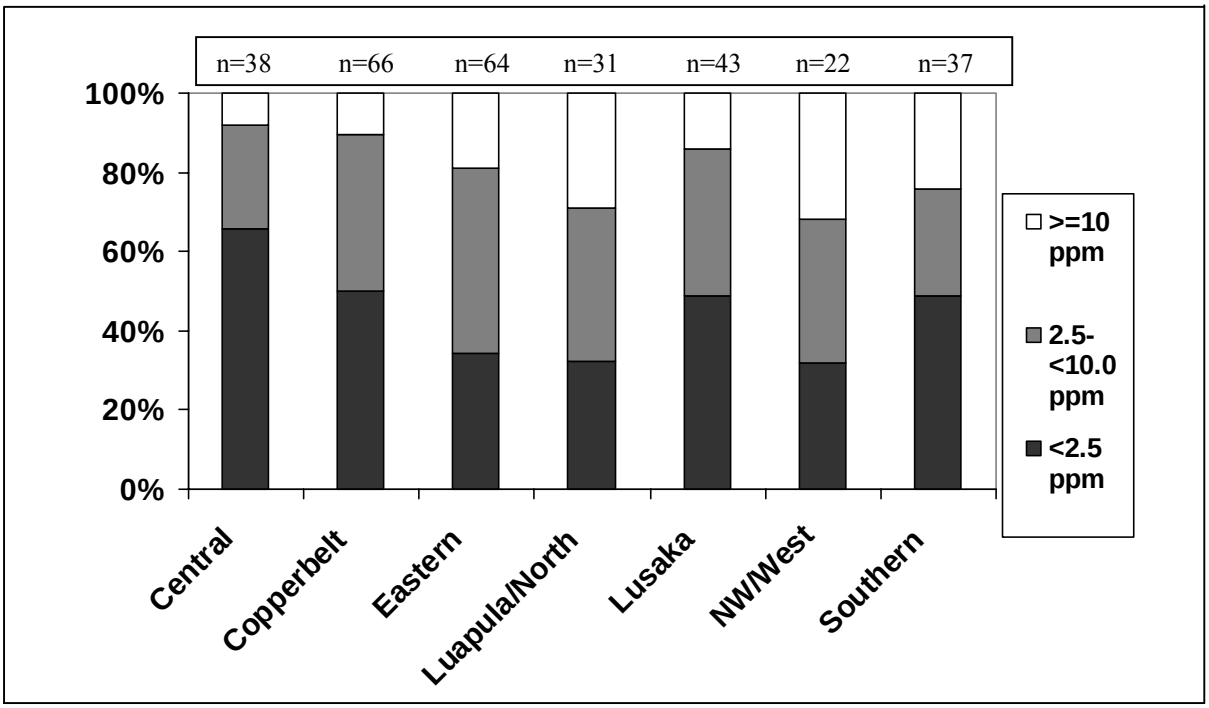


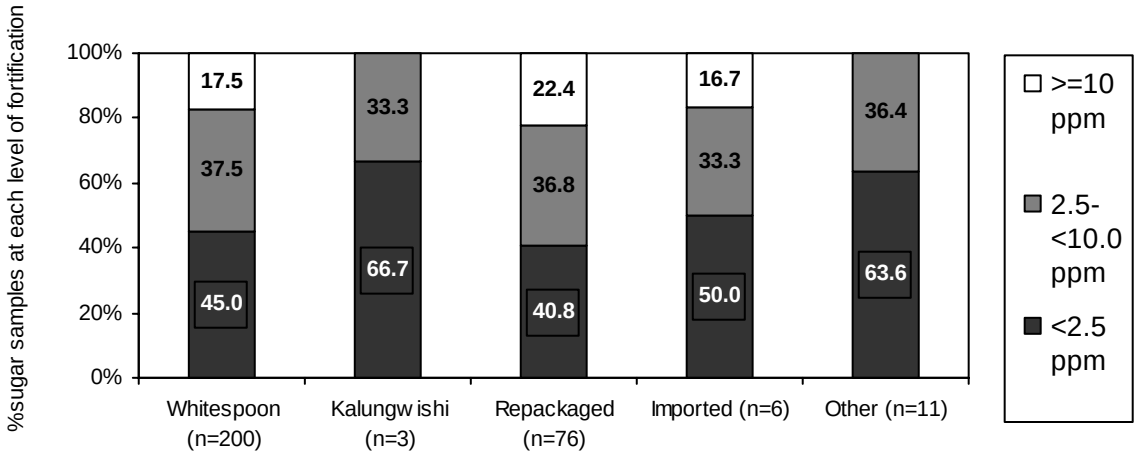
Figure 9.2*:* Adequacy of sugar fortification by Province.



The adequacy of the fortificant levels in sugar was compared across provinces to establish whether there was variation according to the distance the sugar was transported (Figure 9.2). In the Central and Lusaka regions, the nearest areas to the sugar factory, only 8% (Central) and 14% (Lusaka) of sugar was adequately fortified compared with 32% reported from North-Western and

Western regions (more distant areas), suggesting that the amount of fortificant is not related to distance the sugar was transported from the factory.

Figure 9.3: Adequacy of sugar fortification by type of sugar purchased.



The adequacy of sugar fortification by type of sugar showed that among the main types of sugar purchased, Whitespoon sugar and sugar that had been repackaged, there was very little difference in the amount of fortificant present (Figure 9.3). Both had <20% of samples fortified at an adequate level, and more than 40% of samples would be described as having no fortificant present at all. It is probable that the majority of the repackaged sugar was Whitespoon as it was the type of sugar most often used by shopkeepers when repackaging sugar. Also, shop-keepers reported that most sugar is repackaged and sold the same day, so the loss of vitamin A from re-packaging into clear plastic bags is probably minimal. The loss of vitamin A from sugar stored in plastic bags at the household could not be estimated. The imported sugar was similar to the Whitespoon sugar. None of the Kalungwishi and 'other' samples of sugar taken from households was adequately fortified and in both cases more than 60% of the samples would be classified as having no fortificant, (i.e. <2.5 ppm vitamin A).

9.9 Retail sugar

A small number (n = 54) of retail sugar samples were collected from shops nearest to the clusters visited. Only 9.3% (95% CI: 1.5, 17.0) of samples were adequately fortified (≥ 10 mg vitamin A per kg sugar), whereas 40.7 (95% CI: 26.6, 54.9) had levels between 2.5 and <10 mg/kg and 50.0% (95% CI: 33.3, 66.7) had less than 2.5 mg vitamin A per kg of sugar and would be considered to have no fortificant present (Table 9.7). The sugar collected from the retail shops had

fewer adequately fortified samples than those collected at the household. A possible explanation was the household sugar may not have been purchased from the retailers from where sugar was sampled or the retail sugar was from a different manufactured batch than that at the household.

Table 9.7: Adequacy of retail sugar fortification with vitamin A.

Vitamin A/sugar (mg/kg)	Prevalence (n=54) (%)
0 – <2.5	50.0
2.5 – <10.0	40.7
≥ 10	9.3

9.8 Analysis of sugar using spectrophotometric method and fluorescence methods

The household and retail sugar samples were analysed by FDCL in Zambia by two methods: the traditional ultra-violet (UV) spectrophotometric determination of retinol in fortified sugar (Appendix C) and a new method using fluorescence developed by Craft Technologies (Appendix C).

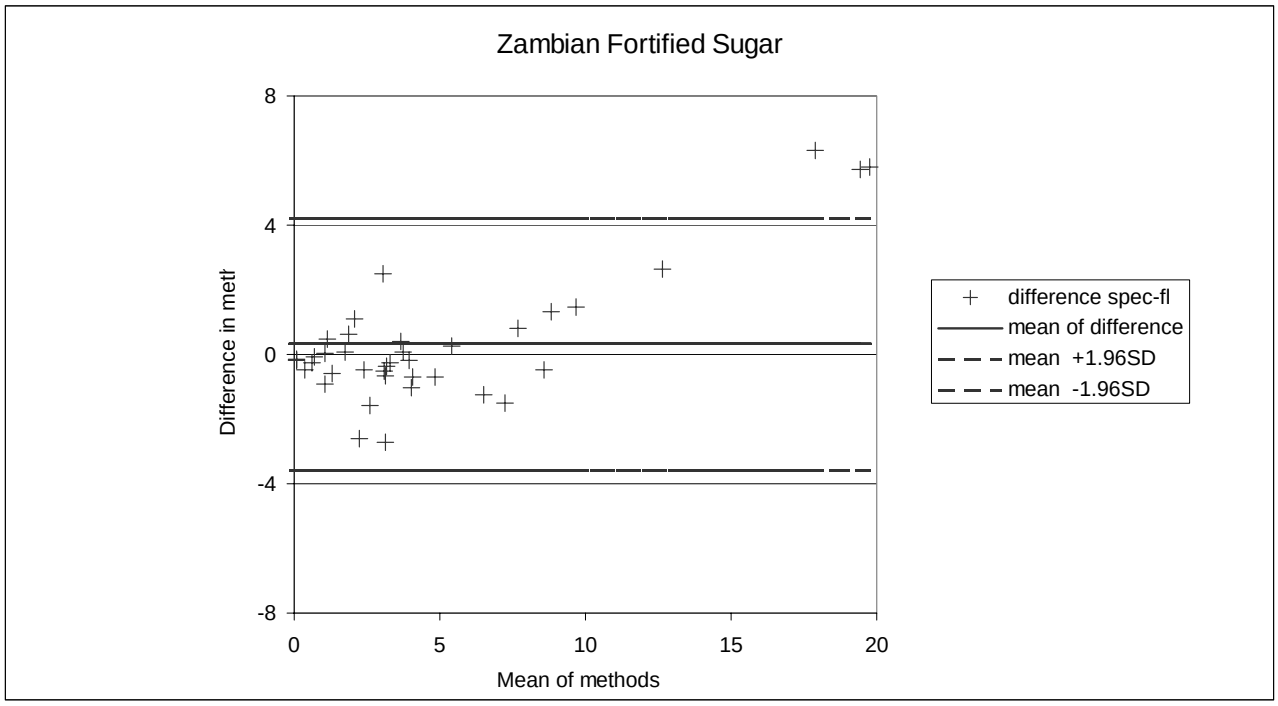
Table 9.8: Comparison of fortificant levels in sugar determined using UV versus fluorescence methods.

Vitamin A Content (ppm)	Fluorescence (n = 305) %	UV (n = 301) %
<2.5	43.0	45.2
2.5 < 5.0	24.9	21.6
5.0 < 10.0	19.0	15.6
10.0 < 15.0	7.5	10.3
15.0 < 20.0	3.6	2.7
20.0 < 30.0	2.0	3.3
>= 30	0.0	1.3

A regression analysis comparing the results of the 2 methods for the analysis of sugar gave an R-squared value of 82%. The Bland and Altman plot (Bland and Altman, 1986) (Figure 9.5) shows the mean of differences of the two methods, and indicates that the fluorescence method tends to give lower results than the spectrophotometric method especially at higher concentrations. At low concentrations there is good agreement between the methods, but for values > 15 ppm, the results from the fluorimetric method are significantly lower (outside the 95% confidence intervals). The

reason for the differences is that the calibration curve used by the fluorimeter tends to level off at higher concentrations. To avoid the bias at the higher concentrations it is necessary to further dilute samples to get them into the lower range where there is good agreement between the methods.

Figure 9.5: Bland and Altman plot of the spectrophotometric compared with the fluorescence method (Bland and Altman,1986).



CHAPTER 10: VITAMIN A STATUS

10.1 Child plasma retinol concentrations

Plasma retinol was measured in all eligible children whose caregiver gave permission for a blood sample to be taken. The overall mean (SD) plasma retinol concentration was 0.71 (0.25) µmol/L (Table 10.1), which was better than in the survey in 1997 when the mean serum retinol was 0.64 µmol/L. In 1997, 65.7% of pre-school children had serum retinol concentrations ≤ 0.7 µmol/L indicating a severe public health problem. In 2003, the independent survey results for July and November gave similar results as 53.3% (95% CI: 44.3, 62.4) (July) and 54.7% (95% CI: 45.3, 64.1) (November) of children had VAD; the overall percentage was 54.1% (95% CI: 46.5, 61.6). The results showed improvement compared with the 1997 results, but still represented a severe public health problem in Zambia (Table 10.1). However, the prevalence of severe VAD, defined as a serum retinol concentration < 0.35 µmol/L (WHO 1996) which was 11.7% in 1997, was halved to 5.0% (95% CI: 2.8, 7.2) in 2003 (Table 10.1).

Table 10.1: Prevalence of severe, moderate and normal vitamin A status and the mean (SD) and median (5, 95th centiles) plasma retinol concentrations (µmol/L) for children aged 6 to 59 months.

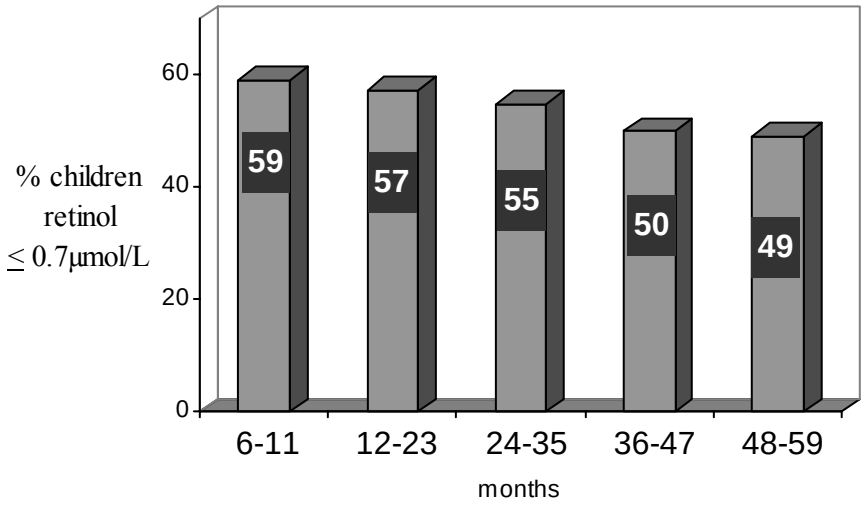
Prevalence of VAD	**1997 (n = 900) %**	**July (n = 317) %**	**November (n = 342) %**	**Combined (n = 659) %**
Severe VAD Retinol < 0.35 µmol/L	**11.7**	**5.7**	**4.3**	**5.0**
Moderate VAD Retinol 0.35 ≤ 0.7 µmol/L	**54.0**	**47.6**	**50.4**	**49.1**
Normal VAD Retinol ≥ 0.7 µmol/L	**34.3**	**46.9**	**45.2**	**45.9**
Plasma retinol concentrations				
Mean retinol µmol/L (SD)	**0.64**	**0.72 (0.26)**	**0.71 (0.24)**	**0.71 (0.25)**
Median retinol µmol/L (5, 95th centiles)	**n/a**	**0.66 (0.34, 1.14)**	**0.67 (0.37, 1.13)**	**0.67 (0.35, 1.13)**

*Weighted results

10.1.1 Prevalence of children with plasma retinol concentrations ≤0.7 µmol/L

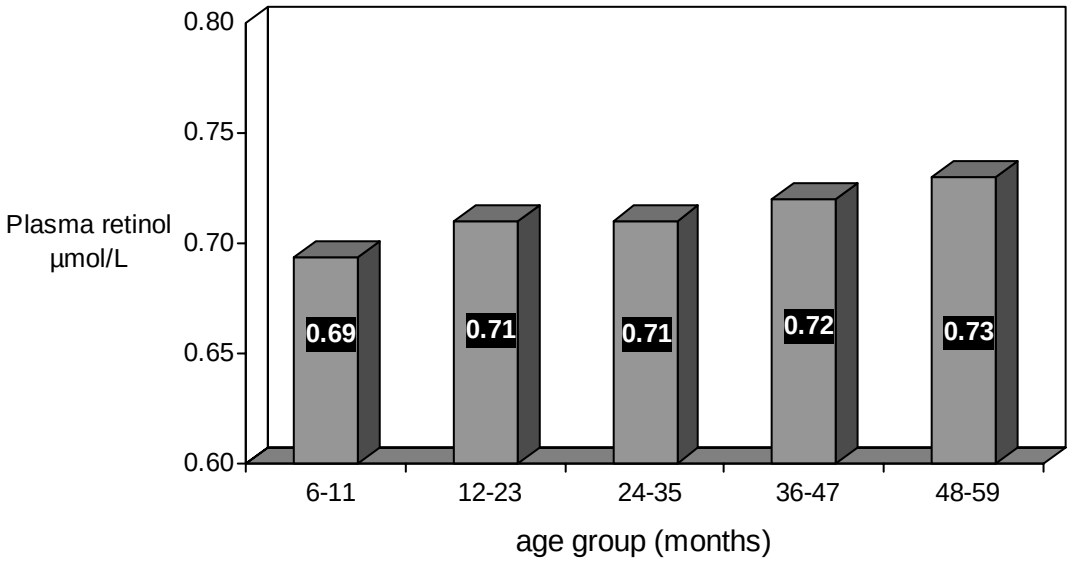
The percentage of children with plasma retinol values ≤ 0.7µmol/L (n = 350) gradually decreased as the children increased in age (i.e. 59% are VAD in the 6–11 month age group compared with 49% in the 48 – 59 month age group) (Figure 10.1).

Figure 10.1: Percentage of children with plasma retinol concentrations ≤0.7 µmol/L
(Weighted analysis)



10.1.2 Age and plasma retinol in children 6-59 months

Figure 10.2: Plasma retinol concentration (µmol/L) by child age group.
(Weighted analysis)



There was an increase in plasma retinol concentration associated with an increase in age (Figure 10.2). The mean retinol concentration in the youngest age group was 0.69 µmol/L compared with 0.73 µmol/L in the oldest group.

10.2 Plasma retinol concentrations in women

Plasma retinol concentrations were measured in women between the ages of 15 and 49 years who were not pregnant and who agreed to give a blood sample. Assuming simple random sampling, the overall mean (SD) plasma retinol concentration for women aged 15 to 49 years, was 1.13 (0.41) μmol/L. A total of 13.4% (95% CI: 9.4, 17.4) of the women had VAD (retinol $\leq$0.7 μmol/L) compared with 21.5 % in the 1997 survey.

CHAPTER 11: FACTORS AFFECTING PLASMA RETINOL LEVELS

11.1 Children 6 to 59 months

11.1.1 Elevated CRP concentrations

It is well known that the presence of an acute phase response will have an adverse effect on plasma retinol concentrations, resulting in a lowering of the retinol concentration in the circulation. The prevalence of children with and without elevated CRP concentrations and classified as being moderately VAD or with normal vitamin A status is shown in Table 11.1. Combining the data from July and November, 71.1% (95% CI: 59.11, 83.09) of children with a CRP > 5 mg/L had plasma retinol concentrations ≤ 0.7 μmol/L, compared with 36.8% (95% CI: 26.1, 47.5) who were VAD but had normal CRP concentrations. ($P < 0.0001$, Variance Estimation Method).

11.1.2 Asymptomatic malaria

Combining the data from July and November, 67.7% (95% CI: 57.7, 77.8) of children were VAD among those positive for malaria and 48.2% (95% CI: 40.8, 55.7) among those negative for malaria children (Table 11.1). The difference in the prevalence of VAD between those with and those without malaria-positive slides was significant ($P = 0.0009$, Variance Estimation Method).

11.1.3 Anaemia

Results similar to those for CRP and malaria were observed for children classified as anaemic, using a haemoglobin concentration < 110 g/L as a cut-off. Overall, 60.4% (95% CI: 52.9, 67.9) of anaemic children were VAD compared with 46.7 % (95% CI: 38.7, 54.8) of non-anaemic children. There was a significant difference in the prevalence of VAD in anaemic and non-anaemic children ($P = 0.0004$, Variance Estimation Method).

11.1.4 Reported morbidity of cough, fever or diarrhoea

Combining the data from the two surveys, 60.7% (95% CI: 51.5, 69.9) of children with fever were VAD compared with 48.3 (95% CI: 39.0, 56.6) without fever ($P = 0.021$, Variance Estimation Method) (Table 11.1).

Table 11.1: Vitamin A status stratified by various morbidity indicators in children aged 6 to 59 months: Data from both surveys combined.

Morbidity indicator	n	*Plasma Retinol ≤ 0.7 µmol/L	*P*
CRP			
>5 mg/L	**117**	**71.1**	**<0.0001**
≤5 mg/L	**177**	**36.8**	
Malaria			
Slide positive	**195**	**67.7**	**0.0009**
Slide negative	**460**	**48.2**	
Anaemia			
Hb < 110 g/L	**329**	**60.4**	**0.0004**
Hb ≥ 110 g/L	**309**	**46.7**	
Fever			
Yes	**304**	**60.7**	**0.021**
No	**353**	**48.3**	
Cough			
Yes	**420**	**56.6**	**0.047**
No	**239**	**49.5**	
Diarrhoea			
Yes	**200**	**65.1**	**0.008**
No	**456**	**48.6**	
Dewormed (July survey only)			
Yes	**207**	**52.3**	**0.233**
No	**107**	**55.6**	

*Results have been weighted to adjust for the sampling design used.

The prevalence of VAD among those with cough was 56.6% (95% CI: 48.4, 64.9) compared with 49.5% without cough (95% CI: 41.1, 57.8; P = 0.047, Variance Estimation Method) (Table 11.1).

The combined data from July and November showed the prevalence of VAD among children with diarrhoea was 65.1% (95% CI: 54.3, 75.9) and was statistically increased compared with those

who had no diarrhoea, 48.6% (95% CI: 40.9, 56.2) (*P* = 0.008, Variance Estimation Method) (Table 11.1).

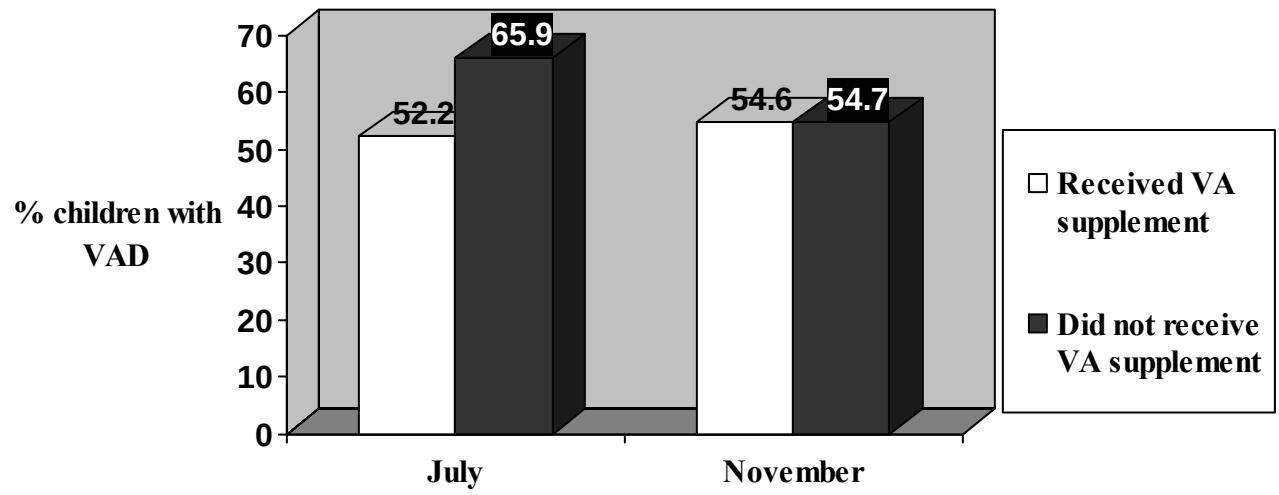
11.1.5 Deworming and the prevalence of VAD (July survey only)

There were no significant differences in VAD between those who had intestinal worms removed and those who did not (Table 11.1). Overall, 52.3% (95% CI: 42.0, 62.6) of children who were dewormed had VAD, and 55.6% (43.7, 67.4) who had not been dewormed had VAD (P = 0.233; Variance Estimation Method) (Table 11.1).

11.1.6 Vitamin A supplementation

The first two bars of the histogram in Figure 11.1 shows the prevalence of VAD among children surveyed in July in relation to whether or not they received a vitamin A capsule in the June CHW. The prevalence of children with VAD was lower among those who received a capsule in June, 52.2% (95% CI: 42.5, 61.9), compared with those who did not receive one, 65.9% (95% CI: 43.1, 88.7; *P* = 0.26) but this difference was not statistically significant. The second two bars of the histogram show the results for November. The percentage of children with VAD who received a vitamin A capsule in June was similar 54.6% (95% CI: 43.7, 65.5), to that of children who did not receive a capsule, 54.7% (95% CI: 41.9, 67.5; *P* = 0.99).

Figure 11.1: Prevalence of vitamin A deficiency (VAD) in children aged 6 to 59 months by whether they received vitamin A supplements in the June CHW



11.1.7 The use and impact of fortified sugar in the household

Fifty-eight percent of caregivers reported buying fortified sugar on a regular basis. There was no difference in the prevalence of VAD in children living in households that provided samples of adequately fortified sugar (≥10 mg/kg) compared with those whose sugar samples were inadequately fortified. Overall, 43.4% (95% CI: 24.4, 62.5) of children who lived in households in which adequately fortified sugar was available were VAD compared with 48.7% (95% CI: 38.1, 59.2) among those from households providing inadequately fortified sugar (P = 0.635). (Results not shown).

11.1.8 Education of the mother

The education of the mothers was associated with the prevalence of VAD in their children. Overall, 61.6% (95% CI: 50.2, 71.9) of children whose mothers had received no formal education or only primary school education had VAD compared with 41.1% (95% CI: 30.0, 52.2) of children whose mothers had received a secondary education or higher (P = 0.004, Variance Estimation Method) (results not shown).

11.2 Non-pregnant women of child-bearing age (15 to 49 years)

11.2.1. Elevated CRP and AGP concentrations

In women with elevated CRP concentrations, the prevalence of VAD was 29.8% (95% CI: 17.3, 42.3), which was significantly higher than in women with normal CRP concentrations 8.6% (95% CI: 5.4, 11.8) (P = 0.0001). Prevalence of VAD in women was not statistically different when data were stratified by AGP. (Table 11.2).

11.2.2. Asymptomatic malaria

Prevalence of VAD in women was not statistically different when data were stratified by malaria (Table 11.3).

11.2.3 Anaemia.

The prevalence of VAD in women who were anaemic was 24.6% (17.3, 31.8) and significantly higher than the prevalence of VAD in women who were not anaemic 8.8% (95% CI: 4.8, 12.9) (P < 0.0001).

Table 11.2: Morbidity indicators and their impact on vitamin A status in women 15 to 49 years of age, data from July and November 2003 combined.

Morbidity indicator	**n**	**Retinol % ≤ 0.7 µmol/L (95% CI)**	***P***
CRP			
> 5 mg/L	**57**	**29.8 (17.3, 42.3)**	
≤ 5 mg/L	**197**	**8.6 (5.4, 11.8)**	**0.0001**
Malaria			
Slide positive	**43**	**16.3 (4.1, 28.4)**	**0.573**
Slide negative	**534**	**12.9 (8.5, 17.3)**	
Anaemia			
Haemoglobin <120 g/L	**167**	**24.6 (17.3, 31.8)**	**<0.0001**
Haemoglobin ≥ 120 g/L	**407**	**8.8 (4.8, 12.9)**	
AGP			
> 1 g/L	**15**	**6.7 (0.0, 21.3)**	**0.870**
≤1 g/L	**50**	**8.0 (0.0, 16.3)**	
Diarrhoea			
Yes	**64**	**9.4 (2.2, 16.5)**	**0.327**
No	**515**	**13.6 (9.3, 17.9)**	
Cough			
Yes	**229**	**15.7 (10.9, 20.6)**	**0.243**
No	**354**	**11.9 (6.6,17.1)**	
Fever			
Yes	**170**	**18.2(12.3, 24.2)**	**0.022**
No	**412**	**11.4 (7.2, 15.6)**	
#Night-blind			
Yes	**14**	**28.6 (0.0, 61.6)**	**0.193**
No	**569**	**13.0 (9.0, 17.0)**	

#Analysis includes only those women who delivered their last child within the previous 36 months.

11.2.4 Reported cough, fever, or diarrhoea

The prevalence of VAD, as defined by a plasma retinol concentration of ≤ 0.7µmol/L, in women with fever was 18.2% (95% CI: 12.3, 24.2) and was significantly higher than in those without fever 11.4% (95 % CI: 7.2, 15.6; *P* = 0.022, Variance Estimation Method) (Table 11.2). Prevalence of VAD in women was not statistically different when data were stratified by cough and diarrhoea.

11.2.5 Education of the woman

The education of the women had no significant impact on the prevalence of VAD. Overall, 14.8% (95% CI: 10.0, 19.6) of women who had received no formal or primary school education had VAD compared with 10.3% (95% CI: 5.1, 15.5) of women who had received a secondary education or higher (*P* = 0.175, Variance Estimation Method). (Results not shown).

11.2.6 Use of oestrogen-based birth control methods, post-partum supplementation or consumption of fortified sugar

There was no significant difference in the prevalence of VAD among women who had been given post-partum supplements or who had lived in a household with adequately or inadequately fortified sugar (Table 11.3). However, there was a significant difference between those

Table 11.3: The effect of oestrogen based birth control, post-partum supplements and fortified sugar on the vitamin A status of women 15 to 49 years of age.

Indicator	**n**	**Retinol % ≤ 0.7 µmol/L**	**Significance *P***
Oestrogen-based birth control			
Yes	**177**	**8.5 (3.7, 13.2)**	
No	**406**	**15.5 (10.6, 20.5)**	**0.049**
Post-partum supplement			
Yes	**210**	**12.4 (5.9, 18.8)**	
No	**355**	**14.4 (9.6, 19.2)**	**0.599**
Retinol fortified sugar available			
Yes (≥10mg/kg)	**39**	**5.1 (0.0, 12.5)**	
No (<10mg/kg)	**199**	**15.1 (8.6, 21.6)**	**0.175**

who used oestrogen-based birth control and those who did not, 8.5% versus 15.5%. The mean plasma retinol concentration of women using oestrogen-based birth control methods was also significantly higher than among those who were not using oestrogen, 1.3 μmol/L (95% CI: 1.2, 1.4)) compared to 1.1 μmol/L (95% CI: 1.0, 1.1) respectively, $P<0.0001$) (not shown**).**

11.3 Correction of plasma retinol concentrations in the presence of infection.

If both CRP and plasma retinol data are collected, then it is possible to exclude those with elevated CRP concentrations, if the percentage of such results in the population is small. Where a large percentage of the population has elevated CRP concentrations, a meta-analysis by Thurnham *et al* (2003) showed that it was possible to correct retinol concentrations for the presence of an acute phase response. The correction indicates what proportion of the VAD is due to an acute phase reaction depressing retinol. Where there is an elevated CRP concentration the equivalent retinol concentration can be multiplied by 1.25. Where there is an elevated AGP concentration, retinol can be multiplied by 1.17.

The meta-analysis (Thurnham *et al*, 2003) indicated that a combination of data for both rapid-responding and slow-responding acute phase proteins could provide the most useful information on the effect of sub-clinical infection on plasma retinol concentrations; hence it is important to correct retinol using the AGP as well as CRP. Unfortunately, the small sample size meant this was not possible in our survey.

11.3.1 Children

The mean plasma retinol concentration for children with elevated CRP concentrations (> 5 mg/L) was 0.61μmol/L in July and 0.63μmol/L in November (Table 11.4). After applying the correction factor for the presence of an elevated CRP concentration, the plasma retinol concentrations for July and November increased to 0.76 and 0.79μmol/L, respectively. In children with normal CRP concentrations, the correction was not applied. Plasma retinol concentrations in these children were 0.88μmol/L in July and 0.79μmol/L in November.

Using the combined results from the July and November surveys, the mean plasma retinol concentrations for 51 children with an elevated AGP was 0.68μmol/L and for 16 children with a normal AGP the mean plasma retinol was 0.87μmol/L. Correction of plasma retinol for elevated AGP resulted in a mean plasma retinol concentration of 0.80μmol/L. (Not shown).

Table 11.4: Correction of plasma retinol concentrations for the presence of infection in children aged 6 to 59 months*

		Plasma retinol July 2003 µmol/L		Plasma retinol November 2003 µmol/L		Plasma retinol Combined data µmol/L	
		Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected
CRP > 5 mg/L	n Mean 95% CI	50 0.61 (0.54, 0.68)	50 0.76 (0.67, 0.85)	67 0.63 (0.56, 0.70)	67 0.79 (0.70,0.88)	117 0.62 (0.57, 0.67)	117 0.77 (0.71, 0.88)
CRP ≤ 5 mg/L	N Mean 95% CI	67 0.88 (0.79, 0.97)	NA	110 0.79 (0.73, 0.86)	NA	177 0.82 (0.76, 0.88)	NA

*Weighted analysis

11.3.2 Correction of retinol concentrations in women using CRP and AGP

The overall mean retinol concentration for the women who also had a plasma sample measured for CRP was 1.12µmol/L. After correcting the retinol concentrations (see sections 3.4) of the samples with elevated concentrations, the mean retinol concentration was 1.17µmol/L. A similar calculation for those women with elevated AGP concentrations resulted in the mean retinol concentration increasing from 1.18µmol/L to 1.22µmol/L.

CHAPTER 12: ANAEMIA

12.1 Children

12.1.1 Anaemia in children aged 6 to 59 months.

A cut-off of < 110 g/L haemoglobin was used to define anaemia in children. In July, 59.3% (95% CI: 51.5, 67.0) and in November 47.0% (95% CI: 39.2, 54.8) of children were anaemic. At both time points, anaemia was a severe public health problem as >40% of children were anaemic (WHO 2001). Overall, 52.9% (95% C.I. 46.7, 59.3) of children had haemoglobin concentrations < 110 g/L in 2003 compared with 65% (95% CI: 62, 67) in the last survey in 1998. About 3.1% of pre-school children surveyed in July were severely anaemic with a haemoglobin concentration <70 g/L. The prevalence of severe anaemia was slightly lower in November (1.2%) (Table 12.1). The mean haemoglobin concentration of the children was 104 g/L in July and 110 g/L in November (Table 12.1).

Table 12.1: Prevalence of anaemia and mean (SD) and median (5, 95th centiles) haemoglobin (Hb) concentrations in children 6-59 months

Prevalence of anaemia	July 2003 n = 353	November 2003 n = 371	Combined n = 724
Severely anaemic Hb 40 - <70 g/L	**3.1%**	**1.2%**	**2.1%**
Anaemic Hb 70 - <110 g/L	**56.2%**	**45.8%**	**50.8%**
Normal Hb ≥ 110 g/l	**40.7%**	**53.0%**	**47.0%**
Haemoglobin concentration (g/L)			
Mean Hb (SD)	**104 g/L (17)**	**110 g/L (15)**	**107 g/L (16)**
Median Hb (5, 95th centiles)	**106 g/L (73, 129)**	**111 g/L (85, 132)**	**109 g/L (77, 130)**

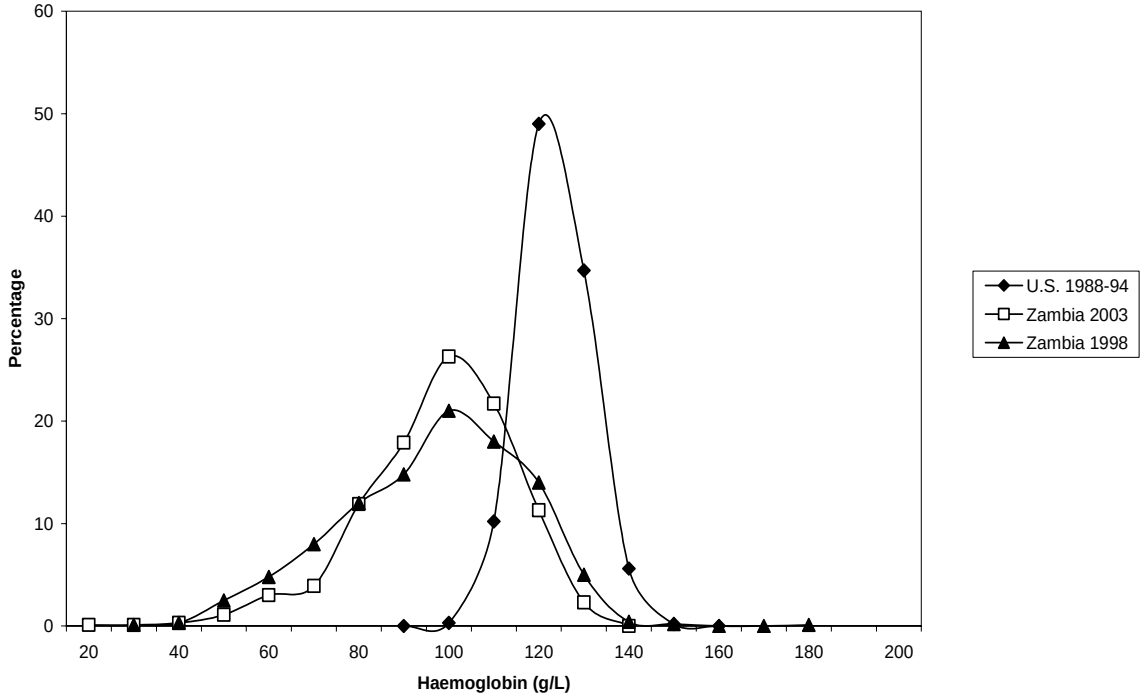
*Weighted analysis

12.1.3 Haemoglobin distribution

Figure 12.1 compares the distribution of haemoglobin concentrations (rounded to the nearest whole number) of Zambian children aged 6 to 59 months with that of American non-iron deficient children 12 to 48 months of age obtained from the National Health and Nutrition Examination Survey (NHANES III 1988-94). Excluding Zambian children younger than 12 months and

greater than 48 months made little difference to the curve. The haemoglobin distribution of Zambian children in 2003 covers a much broader range of concentrations and is skewed to the left compared with the haemoglobin distribution of the US children, and is not substantially different from the haemoglobin distribution of Zambian children of similar age in 1998 (NFNC 1999).

Figure 12.1: Haemoglobin distribution of Zambian children aged 6 to 59 months and American children 1 to 4 years (NHANES III)



12.2 Anaemia in non-pregnant women of child-bearing age (15 – 49 years)

12.2.1 Haemoglobin concentrations in non-pregnant women 15 to 49 years

Overall, 29.1% (95% CI: 24.7, 33.4) of non-pregnant women in Zambia were anaemic (haemoglobin < 120 g/L), compared with 39.1% who were anaemic in 1998 (NFNC 1999). Very few women (0.5%) were severely anaemic (Table 12.2), and the overall mean haemoglobin concentration was 126 (SD 15) g/L (not shown).

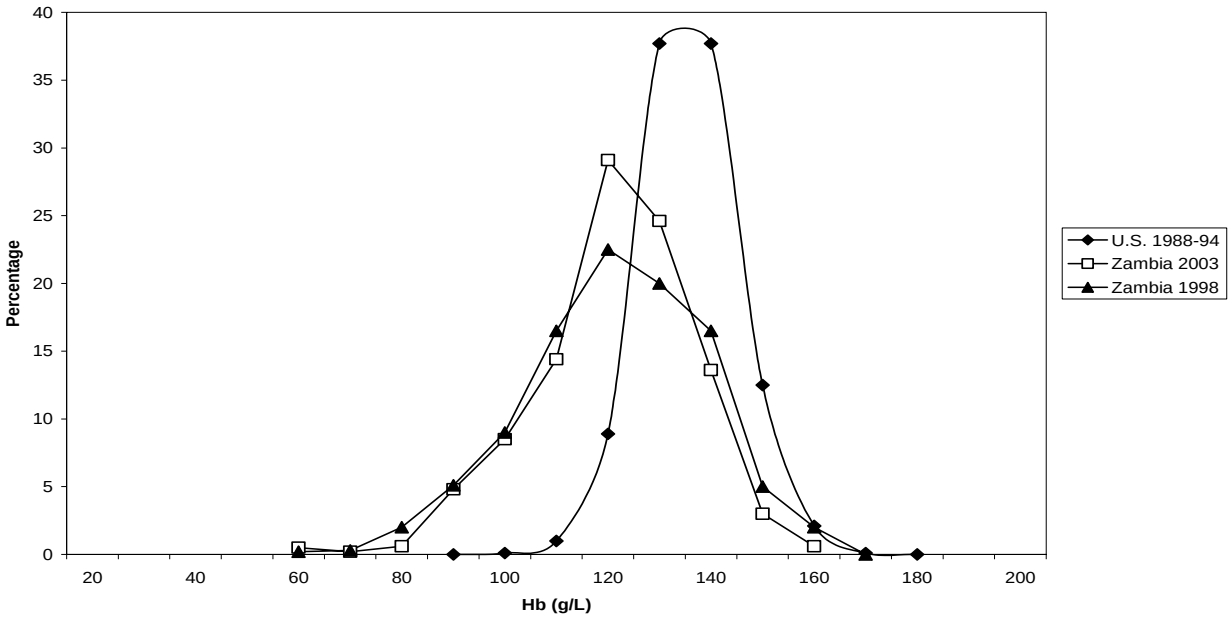
Table 12.2: Prevalence of anaemia in non-pregnant women 15 to 49 years of age.

Anemia	July and November Data combined (n = 623)
Severe (Hb 40– <70 g/L)	**0.5%**
Moderate (Hb 70 – <120 g/L)	**28.6%**
Normal (Hb ≥ 120 g/L)	**70.9%**

12.2.3 Haemoglobin distribution of non-pregnant women

Figure 12.2 compares the distribution of haemoglobin concentrations (rounded to the nearest whole number) of Zambian non-pregnant women 15 to 49 years of age with that of American women 20 to 59 years obtained from the NHANES III 1988-94. The haemoglobin distribution of Zambian women covers a broader range at the lower concentrations compared with the US women. The Zambian women's haemoglobin distribution in 2003 is similar to the haemoglobin distribution in 1998, but there is an increase in the percentage of women (from 20% in 1998 to 28.3% in 2003) with haemoglobin concentration of 130g/L and fewer with low haemoglobin concentrations (2.5% in 1998 and 1% in 2003 between 60 and 80 g/L).

Figure 12.2: Haemoglobin distribution of Zambian women 15 – 49 years and American women 20 – 59 years (NHANES III).



CHAPTER 13: FACTORS AFFECTING HAEMOGLOBIN CONCENTRATIONS

13.1 Children aged 6 to 59 months

13.1.1 Age and anaemia

There was a very significant decrease in the prevalence of anaemia in children ≥ 2 years compared to those < 2 years (Table 13.1).

Table 13.1: Prevalence of anaemia in children by age.

	Prevalence of anaemia					
	July 2003		November 2003		Combined	
Age of child	**n** **%** **(95% CI)**	***P***	**n** **%** **(95% CI)**	***P***	**n** **%** **(95% CI)**	***P***
< 2 years	**142** **68.6%** **(60.7, 76.4)**	**0.005**	**166** **60.9%** **(52.8, 69.1)**	**0.0002**	**308** **64.4%** **(58.7, 70.1)**	**0.0001**
≥ 2 years	**211** **53.1%** **(43.2, 63.0)**		**205** **35.4%** **(24.8, 45.9)**		**416** **44.4%** **(36.4, 52.5)**	

*Weighted analysis

13.1.2 CRP

The presence of an acute phase response causes a lowering of haemoglobin concentrations in the circulation. Among children whose CRP concentrations were elevated (> 5 mg/L), indicating exposure to infection, the percentage of children classified as anaemic in July was 72.9% (95% CI: 54.7, 91.0) compared to 51.2 % (95% CI: 32.8, 69.6) of children with normal CRP. The difference was significant ($P < 0.049$, Variance Estimation Method). In November, there was no significant difference in the prevalence of anaemia between children with or without elevated CRP concentrations ($P = 0.18$; Variance Estimation Method). The results from the combined July and November surveys are presented in Table 13.2.

Table 13.2: The prevalence of anaemia by various morbidity indicators (haemoglobin < 110 g/L) in children aged 6 to 59 months.

Data from July and November 2003 surveys combined.

Morbidity indicator	n	*Anaemia % Hb <110 g/L (95% CI)	*P*
CRP			
> 5 mg/L	**115**	**66.1 (51.3, 80.9)**	
≤ 5 mg/L	**173**	**47.8 (37.9, 57.8)**	**0.037**
Malaria			
Slide positive	**207**	**71.3 (62.5, 80.1)**	
Slide negative	**507**	**45.3 (38.9, 51.6)**	**0.0002**
Fever			
Yes	**330**	**63.1 (56.1, 70.1)**	**0.0000**
No	**391**	**44.4 (38.0, 50.8)**	
Diarrhoea			
Yes	**224**	**59.6 (54.1, 65.1)**	
No	**498**	**50.1 (42.1, 58.0)**	**0.023**
Cough			
Yes	**459**	**53.6 (46.1, 61.0)**	
No	**265**	**52.0 (44.8,59.2)**	**0.684**
Dewormed			
Yes	**398**	**47.9 (40.9, 55.0)**	
No	**314**	**60.4 (52.6, 68.3)**	**0.008**

*Weighted analysis

13.1.3 Asymptomatic malaria

Overall, 71.3% of children with malaria parasites and 45.3% who had no malaria parasites were anaemic. The difference between the two groups was significant (*P* = 0.0002) (Table 13.2).

13.1.4 Reported morbidity of cough, fever or diarrhoea

Fever (*P* = 0.0001) and diarrhoea (*P* = 0.023) were significantly associated with anaemia but cough was not (Table 13.2).

13.1.5 Deworming children

Giving children anthelmintics to remove intestinal worms was not associated with vitamin A status but was associated with anaemia. Combining the data from the two surveys showed children who received anthelmintics had a lower prevalence of anaemia than those who did not, 47.9% vs 60.4% ($P = 0.008$) (Table 13.2).

13.1.6 Vitamin A supplementation

In the July survey, the prevalence of anaemia in children who received vitamin A supplements in June was 57.7% (95% CI: 49.4, 66.1). Among children who did not receive capsules, the prevalence of anaemia was 75.0% (95% CI: 56.9, 93.1). The difference in anaemia prevalence was not statistically significantly ($P = 0.12$). Similarly in November, there was a non-significant difference between the groups ($P = 0.18$) (Results not shown).

13.1.7 Use of fortified sugar in the household

Although 58% of caregivers said they bought fortified sugar on a regular basis (Table 9.2), there was no difference in the prevalence of anaemia between children from a household that provided sugar samples adequately fortified with vitamin A and those from a household that did not. Overall, 46.0% (95% CI: 31.3, 60.7) of children living in households with adequately fortified sugar were anaemic compared with 49.4% (95% CI: 40.1, 58.8) of those households with inadequately fortified sugar ($P = 0.69$) (Results not shown).

13.1.8 Education of the mother

There was no significant difference in the prevalence of anaemia by maternal education. Overall, 54.9% (95% CI: 47.6, 62.2) of children whose mothers had received no formal education or primary school education (Table 4.2) were anaemic compared with 49.0% (95% CI: 39.8, 58.2) of children whose mothers had received secondary education or higher ($P = 0.278$; Variance Estimation Method) (Results not shown).

13.2 Non-pregnant women of child-bearing age (15 – 49 years)

13.2.1. Self-reported and biochemical indicators of morbidity

The presence of an elevated CRP, malaria or reported diarrhoea, fever or cough was not associated with the prevalence of anaemia (haemoglobin < 120 g/L) in the women (Table 13.3). However, women who were VAD were significantly more likely to have anaemia than women who had normal vitamin A status (P = 0.0000, Variance Estimation Method) (Table 13.3).

Table 13.3: The prevalence of anaemia in non-pregnant women 15 to 49 years of age, stratified by morbidity indicators — July and November 2003.

Morbidity indicator	n	Haemoglobin % < 120 g/L (95% CI)	*Significance *P*
CRP			
> 5 mg/L	**57**	**44.4 (25.8, 62.9)**	
≤ 5 mg/L	**195**	**31.5 (22.5, 40.6)**	**0.227**
Malaria			
Slide positive	**45**	**32.3 (21.4, 43.2)**	
Slide negative	**568**	**28.8 (24.3, 33.4)**	**0.482**
VAD			
Retinol < 0.7µmol/L	**255**	**35.6 (28.8, 42.5)**	
Retinol ≥ 0.7 µmol/L	**319**	**24.1 (18.3, 29.9)**	**0.013**
Diarrhoea			
Yes	**68**	**26.1 (15.9, 36.3)**	
No	**551**	**29.6 (25.0, 34.2)**	**0.523**
Cough			
Yes	**248**	**30.8 (23.5, 38.1)**	
No	**375**	**28.5 (23.0, 33.9)**	**0.592**
Fever			
Yes	**182**	**31.8 (23.4, 40.2)**	
No	**439**	**28.5 (22.7, 34.3)**	**0.531**
Night-blind			
Yes	**12**	**6.2 (0.0, 19.0)**	
No	**609**	**29.9 (25.3, 34.4)**	**0.069**

*Weighted analysis

13.2.3 Use of oestrogen-based birth control methods, post-partum supplementation, consumption of fortified sugar or education on anaemia

There was no significant difference in the prevalence of anaemia among women living in households where adequately or inadequately fortified sugar was available (Table 13.4). The use of oestrogen-based birth control or having taken a vitamin A post-partum supplement was associated with a lower prevalence of anaemia in women (Table 13.4).

Table 13.4: The effect of oestrogen based birth control, post-partum supplements and fortified sugar on anaemia in women 15 – 49 years. July and November 2003

Indicator	N	Haemoglobin % < 120 g/L	*Significance P =
Oestrogen-based birth control			
Yes	**194**	**20.6 (14.2, 27.1)**	
No	**429**	**32.9 (27.4, 38.3)**	**0.009**
Post-partum supplement			
Yes	**188**	**20.0 (13.0, 27.0)**	
No	**300**	**34.5 (28.1, 41.0)**	**0.012**
Fortified sugar			
Yes (≥10mg/kg)	**39**	**28.2 (14.1, 42.3)**	
No (<10mg/kg)	**206**	**29.6 (23.4, 35.8)**	**0.854**

*Weighted analysis

CHAPTER 14: SALT IODISATION

The iodine in the salt was measured at the household in the July survey. Of the households surveyed, 227 had salt on the day of the survey and 85% of the samples proved positive for iodine (Table 14.1).

Table 14.1: Results of salt sample iodine test among households who had salt available — July 2003.

Iodine Test	n = 222
Iodine present	**85.5%**
Iodine not present	**14.5%**

CHAPTER 15: DISCUSSION

Overall, the plasma retinol results from the 2-part Zambian National Vitamin A survey show that VAD is still a significant public health problem in Zambian children aged 6 to 59 months. Although severe VAD has been reduced by half, part of the apparent failure of the vitamin A supplementation programme may be attributable to the high levels of sub-clinical infection present in the population, and asymptomatic malaria may be one contributor.

The overall plasma retinol concentration for children 6 to 59 months was 0.71 (SD 0.25) µmol/L. Severe VAD, defined as a plasma retinol concentration < 0.35 µmol/L, was reduced from 11.8% in 1997 to 5.0% in 2003. Retinol concentrations ≤ 0.7µmol/L were found in 54% of children, hence, using the WHO definition (1996) that ≥ 20% of children with plasma retinol concentrations ≤ 0.7 µmol/L is a severe public health problem, VAD is still a public health problem in Zambian children 6 to 59 months of age.

In non-pregnant women 15 to 49 years of age, there has been a significant shift towards eliminating VAD as a public health problem as the percentage of women who had retinol concentrations ≤ 0.7 µmol/L was reduced from 21.5% in 1997 to 13.4% in the 2003 survey.

15.1. Children

15.1.1 Morbidity in children

The presence of malaria parasites in the blood, cough, fever or diarrhoea was associated with a significant decrease in plasma retinol concentration. It is a common observation that the concentration of plasma retinol is lower in the blood of both infants and adults living in the lesser-developed countries of the world. The lower concentrations of plasma retinol are widely accepted to mean that vitamin A status is poorer in those countries than in the developed world (Thurnham *et al,* 2003). It is also well known that the level of exposure to disease is far higher in developing than in developed countries and it is now recognised that disease influences the concentration of plasma retinol. Disease depresses the plasma concentration of retinol-binding protein (RBP), the carrier protein for retinol, reducing the circulating vitamin A and so potentially affects the supply of vitamin A to the tissues during infection (Baeton *et al,* 2004). RBP is a negative acute phase protein, and its synthesis is inhibited during inflammation. It is suggested that the so-called "negative" acute phase proteins are decreased in plasma during the acute phase response to allow an increase in the capacity of liver to synthesise the induced acute phase proteins, e.g. CRP. A

decrease in a carrier protein, such as RBP, reduces the circulating levels of the nutrient they carry, in this case retinol.

The plasma retinol concentrations of Zambian children with acute phase proteins in the normal range were significantly higher (0.82μmol/L) than those with raised acute phase proteins (0.62μmol/L). Combining the data from the July and November 2003 surveys, 70% of children had elevated CRP concentrations (> 5 mg/L) and retinol concentrations ≤ 0.7 μmol/L. Only 37% of children who had normal CRP concentrations were VAD (Table 11.1). Therefore children with an acute phase reaction are much more likely to have VAD than those who do not. A previous meta-analysis showed that it was possible to correct retinol values for the presence of infection (see section 3.4). After using the correction factor, the mean plasma retinol concentration of those who had an elevated CRP increased from 0.62μmol/L to 0.77μmol/L.

Exposure to disease, in particular malaria, is an important factor contributing to the high prevalence of VAD in Zambia. The survey highlights the limitations of using circulating plasma retinol as a measure of vitamin A status in populations that are often exposed to infection. The vitamin A supplements received by the children may have been used by the tissues or may be stored in the liver, but measuring plasma retinol only provides information on the circulating levels. Use of other methods for measuring vitamin A status like the modified dose response, which estimates liver stores (Tanumihardjo *et al*, 1990), or the deuterated-retinol-dilution technique (Haskell *et al,* 1999), which will provide information on total body vitamin stores are used in research but are not routinely used in population surveys. Therefore, plasma retinol measured by HPLC remains the gold standard until alternative methods can be verified.

Presence of malaria parasites in the blood was associated with higher plasma CRP concentrations and lower plasma retinol concentrations. Evidence strongly suggests, however, that micronutrient deficiencies and general under-nutrition increase the burden of malaria morbidity and mortality, so producing a vicious circle. Large numbers of children younger than 59 months die of malaria due to nutritional inadequacies of protein energy, zinc, iron and vitamin A (Caulfield *et al,* 2004). The effect of VAD remains independent of zinc and protein energy deficiencies. Caulfield *et al* (2004) suggest improving the nutritional status of young children in multiple ways, for example, supplementation, fortification, establishing a diverse diet and home gardening may reduce malaria morbidity and mortality and should be considered within a package of interventions to reduce the burden of disease due to malaria.

15.1.2 Vitamin A supplementation of children

Results of these surveys show that 89% of children were reported to have attended the CHW in June and 87% received a vitamin A supplement. Only 66% of caregivers remembered their child receiving a vitamin A supplement in the previous CHW in January. The data were consistent in both the July and November surveys suggesting that the mothers might have remembered if their children attended or did not attend the CHWs in June and January 2003. Data from the 2001-2002 Demographic Health Survey (DHS) in Zambia reported the coverage of vitamin A supplementation was 67% overall, however, there was variation among the provinces with the rate of supplementation being lowest in the Eastern Province (52%) and highest (83%) in the Copperbelt region. Attendance at the January 2003 CHW was poor, but was similar to the coverage reported by the DHS. The reason for the lower numbers of children receiving vitamin A supplements in January 2003 compared with June 2003 was thought to be because of the poor weather conditions of the rainy season making it difficult for mothers to travel with one or more small children on muddy roads. The subsequent CHW in 2003 was moved to December when it was hoped that the slightly better conditions would allow more mothers to attend.

The original hypothesis (section 2.4) was that the prevalence of VAD in aged children 6 to 59 months would be about 30% just after the vitamin A supplementation of children in July and would be about 45% in November just before the next supplementation cycle. In fact, in July the prevalence of VAD was 53.3% and in November was 54.7%. In addition, the mean plasma retinol concentration was expected to be significantly better in July compared with November, but the two concentrations were almost the same as in July and November the concentrations were 0.72 and 0.71μmol/L, respectively. Even after separating the plasma retinol concentrations of healthy children from those with elevated CRP concentrations, there was no difference between the concentrations in July (0.88 μmol/L in healthy children and 0.61 μmol/L in children with an acute phase response) and November (0.79μmol/L in healthy children and 0.63 μmol/L in children with an acute phase response). Why was there no apparent effect of the vitamin A supplementation? Possible explanations are

1. The high-dose supplement was not well absorbed,
2. The high-dose supplement was rapidly excreted or poorly retained after absorption,
3. The daily vitamin A utilisation is high,
4. The high-dose supplement is absorbed and retained in the liver, but is not mobilised for use by extra-hepatic tissue (Miller et al, 2002).

Because plasma retinol will only measure circulating levels so it is not possible to provide an answer. To investigate what has happened to a high-dose vitamin A supplement it will be

necessary to estimate the absorption and retention of high-dose supplements, the daily utilisation rate of vitamin A, acute phase proteins and total body pool size of retinol before and after administration of a high-dose supplement. A study to do this has been proposed (section 16).

15.1.3 Child feeding

Exclusive breastfeeding for the first 6 months is known to help in protecting the children from infection, including HIV transmission, enabling the children to build up more vitamin A liver stores to help them through the vulnerable weaning period (Miller *et al* 2002). Based on the Zambian 2003 survey, 56% of infants were given some type of complementary food before the age of 6 months and only a third of Zambian women exclusively breastfed their children for 6 months. A recent joint statement from UNICEF and WHO (2004) recommends the optimal method of infant feeding for women who are HIVnegative or do not know their status should be: exclusive breastfeeding for 6 months. Thereafter, infants should receive safe and nutritionally adequate complementary foods with continued breastfeeding up to 2 years of age or beyond. Approximately 5 to 20% of infants born to HIV-infected women will be infected through breastfeeding (De Cock *et al* 2000). Given the need to reduce HIV transmission to infants while minimising the risks of other causes of morbidity and mortality, UNICEF and WHO state that: "when replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all breastfeeding by HIV-infected mothers is recommended. Otherwise, exclusive breast feeding during the first months of life which should be discontinued as soon as it is feasible."

15.2 Women 15 – 49 years

15.2.1 Morbidity

Overall, there was not as much infection reported for women compared with their children. Seven percent of women had malaria positive slides, 10% had diarrhoea, more than one-quarter had fever and 41% had cough. Fever, elevated CRP and anaemia were all associated with a higher prevalence of VAD (section 11.2.1). The presence of malaria, reported diarrhoea or cough was not associated with an increase in the prevalence of VAD (Table 11.4). The overall mean plasma retinol concentration of women who had a plasma sample measured for CRP was 1.12µmol/L and after correcting the retinol concentrations using the meta-analysis data, the mean retinol concentration was 1.17µmol/L. By comparison the median retinol concentration of US women, aged 20 to 59 years, measured as part of NHANES III was 1.71 µmol/L (Ballew *et al* 2001) and the mean retinol concentration for Scottish women aged 16 to 44 years in the UK was 1.80 (SD

0.04) µmol/L (The Scottish Health Survey,1998). As with the retinol data for the children, the plasma retinol concentrations for the women were not adjusted for the presence of chronic infection,which might have increased the mean overall retinol concentration.

In 2003, 2.3% of women were reported to be night blind compared with 11.6% in 1997. In 1997, women were only asked if they had difficulty seeing at night and not whether they had difficulties seeing in the day, which possibly accounts for the large difference in the results between 1997 and 2003.

15.2.2 Vitamin A supplementation

Post-partum vitamin A supplementation coverage in Zambia was 37%. Among women, vitamin A supplements were associated with a lower prevalence of VAD, and anaemia (see section 13.2.3). Previous studies have shown that post-partum supplements can improve liver stores of women and increase vitamin A concentrations in breast milk, neither of which was measured in the survey (Ross 2002). Improved vitamin A status in women will improve the vitamin A status of their infants via breast milk and allow the infant to build up liver stores of vitamin A to help them through the vulnerable weaning period (Ross, 2002).

15.3 Oestrogen-based birth control methods

The use of oestrogen-based birth control methods was significantly associated with higher mean plasma retinol concentrations (section 11.2.4). This effect was also noted in the NHANES III data where non-Hispanic whites, non-Hispanic blacks and Mexican women 14 years of age or older taking oestrogen had mean serum retinol concentrations of 2.19, 1.98 and 2.04 µmol/L respectively compared with 1.85, 1.68 and 1.62 µmol/L respectively in the same population groups not taking oestrogen (Ballew *et al* 2001). The interaction of oestrogen with plasma retinol is not understood but is thought to be at the cellular level via cellular retinoic acid binding protein II moving retinoic acid to its nuclear receptors (Amatayakul *et al* 1989; Schweigert, 2001; Li and Ong, 2003).

15.4 Sugar

15.4.1 Sugar purchase and consumption

Approximately 59% of households reported buying fortified sugar on a regular basis. Both money and seasonality were constraining factors on how much sugar was bought. Over half of households bought more sugar at the beginning of the month, which suggests more money was available in the household at that time, and when money was limited, sugar was one of the items

not purchased. There was conflicting data about seasonality affecting the amount of sugar purchased. In the July survey, about half of respondents reported consuming less sugar during the rainy season. In November, only a third of respondents reported consuming less sugar in the rainy season. The discrepancy may be due to the respondents forgetting how much sugar they bought in the last rainy season as the November survey was held at the end of the dry season.

About a third of children were reported to drink tea with sugar, 40% to eat porridge with sugar and a small proportion to eat cereal with sugar. Similarly, about 40% of women reported drinking tea with sugar, 17% eating porridge with sugar and 10% eating cereal with sugar. In households that reported consuming fortified sugar, however, the prevalence of VAD and anaemia was not different from households that reported having no sugar. The women and children who lived in households that had fortified sugar may not have consumed enough sugar to have had a beneficial effect on vitamin A status. In addition, sugar was not consistently fortified at the correct level. Sugar fortification programmes in Guatemala, Nicaragua and other South American countries are having positive effects on the prevalence of VAD (Dary *et al,* 2005 in press). The Zambian sugar vitamin A fortification programme probably could have an impact on vitamin A status in those who regularly eat sugar that is consistently adequately fortified, despite the higher rates of infection in Zambia compared with South America.

15.4.2 Fortification of sugar

Overall, about 83% of sugar samples collected from the households had < 10 mg retinol equivalents vitamin A per kg sugar, which is below the minimum legal level of fortification. In addition, 90% of the retail sugar collected from shops near to the survey clusters also failed to have adequate fortificant levels (see section 9.7). At present there is no adequate monitoring system in place in Zambia that will identify when and where there are problems in the sugar fortification programme.

In September 2003 a team from MOST Washington and INCAP Guatemala visited Zambia Sugar, the principle sugar producer, and found problems with the fortification process at the factory. The problems have been addressed, but it is necessary to instigate a monitoring system to ensure the sugar is produced with fortificant added at the correct level and that there are no significant losses in the vitamin A content of the sugar between production and consumption at the household level.

15.4.3 Methods for assessing vitamin A in sugar

The two methods for assessing vitamin A in sugar gave good agreement at lower concentrations of vitamin A but above 15 mg RE/g the fluorescence assay gave significantly lower values. The reason for the difference is that the calibration curve for the fluorimeter tended to level off at higher concentrations. To use the fluorescence method it will be necessary to further dilute sugar samples of high concentration to get them into the range where the method is accurate.

15.5 Anaemia

15.4.1 Children

Anaemia is an indicator of both poor nutrition and poor health. Iron deficiency anaemia (IDA) is the most severe form and often the prevalence of anaemia, as measured by haemoglobin, is used as a proxy for IDA. In areas where iron deficiency may not be the major cause of anaemia and the aetiology may be more complex, the use of haemoglobin as a proxy for IDA is not appropriate. Zambia is such an area where the aetiology may be complex because of the exposure to infections such as malaria and helminth infections and deficiencies in other nutrients. As part of the 2003 surveys only haemoglobin was used as a measure of iron status and hence can only be interpreted as a measure of anaemia. Combining the data from the two surveys, anaemia (haemoglobin < 110 g/L) was found in 53% of children aged 6 to 59 months. A higher proportion of children younger than 24 months (64%) were anaemic compared with the older age groups (44%), and the difference in prevalence was significant.

The distribution curve of haemoglobin concentrations in 2003 showed a slight improvement compared with that obtained in 1998. There was a higher percentage of children with haemoglobin concentrations in the 9 to 11 g/dL range and an improvement in the prevalence of children with the lower concentrations of haemoglobin (Figure 12.2). Compared with the US NHANES III data, the distribution of the Zambian haemoglobin concentrations is still shifted to the left and is more widely spread.

15.5.1 Morbidity and anaemia

The prevalence of anaemia in children was associated with the presence of an elevated CRP concentration, malaria parasites in the blood, VAD, cough, fever and not being dewormed. Common infections, especially those which are chronic and/or recurrent may impair haematopoiesis and consequently cause anaemia (Macdougall and Cooper, 2002). In Zambia, many children showed evidence of malaria parasitaemia, even if they were not clinically sick. The presence of the malaria parasite triggers an acute phase response that in turn has negative effects

on haemoglobin concentrations (Chang and Stevenson, 2004). Other infections had similar negative effects on haemoglobin concentrations. HIV/AIDS is another cause of anaemia and anaemia is recognized as an independent risk-factor for early death among HIV-infected individuals (Moore 1999). Although HIV/AIDS is a major public challenge in Zambia, especially among individuals aged 15 to 49 years, we did not test for it. However, we do recognize the importance of its impact.

15.5.2 Vitamin A supplements, vitamin A fortified sugar and anaemia

Neither vitamin A supplements given to children nor the use of fortified sugar by the household was associated with the prevalence of anaemia.

15.5.2 Anaemia in non-pregnant women

Anaemia was found in 30% of women but did not appear to be associated with the presence of an elevated CRP or reported diarrhea, fever or cough, or the presence of a malaria positive slide. Demographics and education were also not related. Women with VAD as assessed by plasma retinol were more likely to be anaemic. The use of oestrogen-based birth control methods or having taken a post-partum vitamin A supplement was associated with a significantly lower prevalence of anaemia. Perhaps oestrogen has some kind of effect on erythropoeisis at the molecular level but no data were available to confirm this. Vitamin A supplements have been shown previously to have beneficial effects on anemia (Bloem *et al* 1990; Suharno *et a*l 1993).

Chapter 16: Recommendations.

Following the dissemination of the results from the Zambian vitamin A surveys, a round table discussion was held in Lusaka on 9 September 2004. The discussion was attended by members of NFNC, MoH, MOST Zambia, Care Zambia and CDC. The results from the vitamin A surveys were discussed and recommendations for the future were proposed:

Vitamin A

Child health weeks (CHW)

To strengthen the implementation of CHWs at community and district level, there is need to

- Encourage community innovation,
- Encourage community participation to take ownership,
- Encourage private health facilities to take part,
- Improve coverage of vitamin A supplementation in all districts,
- Use advocacy to improve programme support from policy makers.

Child–feeding practices should:-

- Promote exclusive breast feeding in the first 6 months for women who are HIV negative or who do not know their status. For HIV positive women, when replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all breastfeeding is recommended. Otherwise, exclusive breast feeding is recommended during the first months of life and should be discontinued as soon as it is feasible.
- Thereafter infants and young children should receive safe and nutritionally adequate complementary foods with continued breastfeeding up to 2 years of age, where appropriate (see above).
- Explore low dose (50,000 IU) early supplementation, as a way of ensuring an adequate supply of vitamin A to infants during the first six months. The proposed early vitamin A supplementation can be linked to routine immunization.

Women

Improve the postpartum vitamin A capsule supplementation programme by:-

- Linking supplementation to BCG immunisation of children within 6 to 8 weeks of delivery
- Distribution of the vitamin A in maternity wards

Food fortification

- Fortification of sugar with vitamin A is an important strategy to improve the vitamin A status of the Zambian population, and it is essential to strengthen enforcement and monitoring of the regulation of the statutory instrument (No.155) to ensure adequate and consistent vitamin A fortification. The statutory instrument states that packaged sugar is sold with a minimum fortification level of 10 mg per kg sugar.
- Explore vitamin A fortification of other commonly consumed foods (e.g. maize meal, cooking oil and milk).

Dietary diversification

- Consumption of vitamin A–rich foods is one of the long-term strategies that can improve vitamin A status, therefore, the promotion, production and consumption of vitamin A–rich foods, such as red palm-oil and yellow fleshed sweet potato, should be encouraged.

Infection

- Distribution of treated bed-nets should continue as part of the CHW programme. Heavy disease burden, especially through malarial parasites, depresses circulating concentrations of retinol.
- Deworming should continue as part of CHW.
- Other public health interventions should be encouraged (e.g. clean water, sanitation).
- To reduce exposure to infection and improve both vitamin A and iron status, education about reducing exposure to infection should be promoted (e.g. use of bednets, hand washing etc.).

A proposal was put forward for an intensive study on a small group of children 3 to 4 years of age to understand why high-dose capsules have had little impact on plasma retinol in children. The study will help our understanding of the absorption, retention and long-term impact of high-dose vitamin A supplements in children.

Iron

To strengthen the programme to reduce anaemia the following recommendations were proposed:

- Targeted interventions to provide iron supplements to the most vulnerable population groups (e.g. children < 5 years of age and women, especially pregnant women),

- Food-based approaches to increase iron intake through food fortification (e.g. flour). Continuing to include a deworming programme as part of CHW, as the use of anthelmintics was associated with a lower prevalence of anaemia in children,
- Continue to include the distribution of treated bed-nets as part of the CHW programme. Use of bednets will reduce the number of mosquito bites, which will reduce sub-clinical and clinical malaria, resulting in a reduction in the prevalence of anaemia,
- Education to increase awareness and knowledge among health care providers and the general public on the health risks associated with anaemia,
- Dietary diversification

REFERENCES

Africa Fighting Malaria (2002) http://www.fightingmalaria.org/index.php.

Amatayakul K, Underwood BA, Ruckphaopunt S, Singkamani R, Linpisarn S, Leelapat P, and Thanangkul O(1989). Oral contraceptives: effect of long-term use on liver vitamin A storage assessed by the relative dose response test. American Journal of Clinical Nutrition 49**:** 845 - 848.

Baeton JM, Richardson BA, Bankson DD, Wener MH, Kreiss JK, Lavreys L, *et al* (2004). Use of serum retinol-binding protein for prediction of vitamin A deficiency: effects of HIV-1, protein malnutrition and the acute phase response. American Journal of Clinical Nutrition 79:218-225.

Ballew C, Bowman BA, Sowell AL and Gillespie C (2001). Serum retinol distributions in residents of the United States: third National Health and Nutrition Examination Survey, 1988–1994. American Journal of Clinical Nutrition 73:586-93.

Bland JM and Altman DG (1986). Statistical methods for assessing agreement between two methods of clinical measurement. Lancet i 307-310.

Bloem MW, Wedel M, van Agtmaal EJ, Speek AJ, Saowakontha S and Schreurs WH (1990). Vitamin A intervention: short-term effects of a single, oral, massive dose on iron metabolism. American Journal of Clinical Nutrition 51:76-79.

Chang KH and Stevenson MM (2004). Malarial anaemia: mechanisms and implications of insufficient erythropoiesis during blood-stage malaria. International Journal of Parasitolology 34(13-14):1501-16

Cogswell M (1999) Distribution of haemoglobin concentrations in non-iron deficient American populations from NHANES III data (1988-94): children 1–4 years and women 20–59 years. Personal communication from unpublished data.

Coutsoudis, A (2005). Breastfeeding and HIV. Best Practice and Research Clinical Obstetrics and Gynaecology 19:185-196.

Coutsoudis A, Pillay K, Kuhn L, Spooner E, Tsai WY, Coovadia HM; South African Vitamin A Study Group (2001). Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: prospective cohort study from Durban, South Africa. AIDS 15(3):379-87.

Craft NE, Estes JE, and Furr HC (2004) Use of the CRAFTi portable fluorometer to measure vitamin A in fortified foods and blood. Abstract T3. IVACG Lima, Peru.

Dary O, Martinez C, Guamuch M (in press). Sugar with vitamin A in Guatemala: A successful food fortification program, but still at risk.

De Cock KM, Fowler MG, Mercier E, de Vincenzi I, Saba J, Hoff E, Alnwick DJ, *et al* (2000). Prevention of mother-to-child HIV Transmission in resource-poor countries. Translating research into policy and practice. Journal of the American Medical Association. 283:1175-1182.

Expert Scientific Working Group (1985). Summary of a report on assessment of the iron nutritiojnal status of the United States population. American Journal of Clinical Nutrition 42:1318-30.

Fisherman SM, Christian P and West KP (2000). The role of vitamins in the prevention and control of anaemia. Public Health Nutrition 3:125-150.

Gabay C and Kushner I (1999). Acute phase proteins and other systemic responses to inflammation. The New England Journal of Medicine 340:448-54.

Haskell MJ, Mazumder RN, Peerson JM et al (1999). Use of the deuterated-retinol-dilution technique to assess total body vitamin A stores of adult volunteers consuming different amounts of vitamin A. American Journal of Clinical Nutrition 70:874-880.

Linkages (2004) AED Washington www.linkagesproject.org

Li XH, Ong DE. (2003) Cellular retinoic acid-binding protein II gene expression is directly induced by estrogen, but not retinoic acid, in rat uterus. Journal of Biological Chemistry 278(37):35819-25.

Macdougall IC and Cooper A (2002). The inflammatory response and epoetin sensitivity. Nephrology Dialysis Transplantation 17 (supplement 1): 48-52.

Micronutrient Initiative (MI) and Progamme for Appropriate Technology in Health (PATH) (2000) Fortification Rapid assessment tool and guidelines. http://www.micronutrient.org/resources/publications/frat.pdf

Miller M, Humphrey J, Johnson E, Marinda E, Brookmeyer R and Katz J (2002). Why do children become vitamin A deficient? Journal of Nutrition 132 supplement: 2867S-2880S.

Moore RD (1999). Human immunodeficiency virus infection, anemia, and survival. Clinical Infectious Diseases 29(1):44-9.

National Diet, Nutrition and Dental Survey of Children Aged 1 1/2 to 4 1/2 Years, 1992-1993 United Kingdom. (MAFF and MoH transferred to Food standard Agency 2000) at http://www.data-archive.ac.uk/

National Food and Nutrition Commission (1997) National survey on vitamin A deficiency in Zambia: A random cluster study for children (0–5 years) and mothers attending national immunisation days in august 1997. Report prepared by C Luo and C Mwela.

National Food and Nutrition Commission (1999) National Baseline survey on prevalence and aetiology of anaemia in Zambia: a random cluster community survey involving children, women and men. Report prepared by C Luo, C Mwela and J Campbell.

Pilch, S.M., ed**.** 91987). Analysis of vitamin A data from the health and nutrition examination surveys. Journal of Nutrition*,* 117: 636-640.

Ross DA (2002). Recommendations for vitamin A supplementation. Journal of Nutrition 132 supplement: 2867S-2880S

Schweigert FJ. Siegling C. (2001). Immunolocalization of retinol-binding protein, cellular retinoic acid-binding protein I and retinoid X receptor beta in the porcine reproductive tract during the oestrous cycle. Reproduction, Fertility, & Development. 13(5-6):421-6.

Sight and Life Manual on Vitamin A Disorders (VADD) (2001). Other Effects of vitamin A deficiency. Chapter 7. Editors DS McLaren and M Frigg. Task Force SIGHT AND LIFE, Basel, Switzerland.

Sommer A (1995) Vitamin A deficiency and its consequences. A field guide to detection and control, 3rd edition, World Health Organisation, Geneva, Switzerland.

Sommer A and Davidson FR (2002). Assessment and control of vitamin A deficiency: The Annecy Accords. Journal of Nutrition 132: 2845S-2850S.

Sommer A and West KP Jr (1996). Vitamin A deficiency: Health, Survival and vision. Oxford University Press New York.

Suharno D, Muhilal, Karyadi D, West CE, Hautvast JGAJ and C. E. West (1993) Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. The Lancet 342:1325-1328.

Tanumihardjo SA, Koellner PG and Olson JA (1990). The modified relative-dose response assay as an indicator of vitamin A status in a population of well-nourished American children. American Journal of Clinical Nutrition 52:1064-1067.

The Scottish Health Survey 1998, Volume 1. A survey carried out on behalf of The Scottish Executive Department of Health. Edited by A Shaw, A McMunn and J Field. Joint Health Surveys Unit, The National Centre for Social Research and Department of Epidemiology and Public Health, UCL. Published 2000.

Thurnham DI, McCabe GP, Northrop-Clewes CA and Nestel P (2003). A meta-analysis of 15 studies to quantify the effects of sub-clinical infection on plasma retinol concentration and to assess the prevalence of vitamin A deficiency. The Lancet 362:2052-8.

UNAIDS (2002) www.unaids.org/en/geographical+area/by+country/zambia
www.mongabay.com/external/AIDS_stats/UNAIDS/Zambia

UNICEF (2004) http://www.unicef.org/emerg/southernafrica/

WHO/Nut 96.10 Micronutrient series: Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes.

WHO (2001) Iron deficiency anaemia: Assessment, prevention and control. A guide for programme managers. WHO/NHD/01.3. Geneva, Switzerland.

World Health Organisation (2002) http://www.who.int/nut/vad.htm

WHO (2003) Global strategy for infant and young child feeding. Geneva, Switzerland. http://www.unicef.org/media/files/gs_infant_feeding_text_eng.pdf

WHO (2004) World Health Report http://www.who.int/hiv

WHO/UNICEF (2004). UNICEF and WHO call for stronger support for the implementation of the Joint United Nations HIV and infant feeding framework. New York & Geneva.

Appendix A

Code of Ethics for Survey Personnel

Code of Ethics for Survey Personnel

Upon acceptance and signing of your contract, you are required to carry out your duties in a professional manner. All questions on the questionnaires should be completed with the appropriate answers given by the respondent. Do not fill in an answer based on an assumption or one made up by any of the team members in hindsight.

The lab personnel should ensure the correct protocol is followed and the required samples and measures are collected and recorded on the questionnaire.

Please respect the confidentiality of all the survey participants. Remember, the quality of the data is the essential part of the survey, so it is important that you take time to ensure to collect high quality data.

I agree to participate and the study and abide by the code of ethics described above:

Print your full name

_____

Sign your name and date

Return signed form to Eva Politzer or Christine Clewes before Wednesday, October 29, 2003.

Appendix B
Informed consent forms

Informed Consent Information for Mothers and Female Caregivers 15-49 years

National Vitamin A Assessment in Zambia

The Ministry of Health is doing a study to find out if the people of Zambia are getting enough vitamin A and iron in the food they eat. Eating enough vitamin A and iron is important. They help to keep us healthy.

By chance, your family was chosen to be in this study. By being in the study, you are helping us to improve the health of the people of Zambia. If you agree to be in the study, we will need about 45 minutes of your time.

We want to know if Zambia's program to add vitamins to food or give them in capsules is working.

If you agree, we will ask you some questions about your health and about the food you eat. We will also ask you about the health of one of your children under 5 years old and the food that he or she eats. This child will also be chosen by chance.

We will also ask you to give us about one cup of sugar that you use to make family meals. In exchange, we will give you a 1-kilo packet of sugar. The sugar that you give us will be tested to find out how much vitamin A it has in it.

We will then ask for a small sample of blood from your finger. We will also ask for blood from the finger of the child chosen for the survey. The blood will be measured for tests related to vitamin A, hemoglobin levels and to check for malaria. No other tests will be done on your blood or your child's blood.

When we prick your finger to take blood, you may feel slight pain for a few seconds. There should be no other risk or pain.

We can give you the results of the blood hemoglobin test before we leave. All other tests must be done in a laboratory, so we will not be able to give you those results. If our tests show that you are low in iron, we will refer you to the nearest health center. A referral will also be given to your child if his/her hemoglobin is low.

If you do not want to be in this study you are free to say "no". There will be no bad affect on you or your family. If you do agree to be in the study, you do not have to answer any question that you do not want to answer. If you want to stop the study you may do so at anytime. Neither your name nor your child's full name will be written down. No names will be used when reporting about this survey

People in this study have certain rights. If you have questions about your rights, you may contact Dr. Chipepo Kankasa, University Teaching Hospital, Department of Paediatrics, P.O. Box RW1, Lusaka, telephone 01-252662. If you have any health problems or concerns related to this study you may contact your local District Health Management Board.

Consent: (signature or thumb print required on appropriate line)

I understand what this study involves, and:

I agree that I will be a part of the study Yes No

I agree that my child _____ will be part of the study Yes No
[print name of child]

Signature or thumbprint: _____

Printed name: _____

Date: _____

Informed Consent Information for Male Caregivers and Women < 15 or > 49 Years
National Vitamin A Assessment in Zambia

The Ministry of Health is doing a study to find out if the people of Zambia are getting enough vitamin A, iron, and iodine in the food they eat. Eating enough vitamin A and iron is important. They help to keep us healthy.

By chance, your family was chosen to be in this study. By being in the study, you are helping us to improve the health of the people of Zambia. If you agree to be in the study, we will need about 35 minutes of your time.

We want to know if Zambia's program to add vitamins to food or give them in capsules is working. We also want to know if the new way of measuring vitamin A in food works well.

If you agree, we will ask you some questions about the health your child that we have selected by chance and the food that he or she eats.

We will also ask you to give us about one cup of sugar that you use to make family meals. In exchange, we will give you a 1-kilo packet of sugar. The sugar that you give us will be tested to find out how much vitamin A it has in it.

We will then ask for a small sample of blood from the finger of the child chosen for the survey. This will blood will be measured for tests related to vitamin A, iron levels and malaria. These are the only tests that we will do on this blood.

When we prick your child's finger to take blood, he/she may feel slight pain for a few seconds. There should be no other risk or pain.

We can give you the results of the iron test done on this blood before we leave. All other tests must be done in Lusaka, so we will not be able to give you those results. If our tests show that your child is low in iron, we will refer him/her to the nearest health centre.

You do not have to be in this study. You are free to say "no" and there will be no bad affect on you or your family. If you do agree to be in the study, you do not have to answer any question that you do not want to answer. If you want to stop the study you may do so at anytime. Neither

your name nor your child's full name will be written down. No names will be used when reporting about this survey

People in this study have certain rights. If you have questions about your rights, you may contact Dr. Chipepo Kankasa, University Teaching Hospital, Department of Paediatrics, P.O. Box RW1, Lusaka, telephone 01-252662. If you have any health problems or concerns related to this study you may contact your local District Health Management Board.

Consent: *(if consent given, signature or thumb print required on appropriate line)*

[ ] I understand what this study involves, and I agree that my child _____ may take part.
[print name of child]

[ ] Consent not given

Signature or thumbprint of father or other caregiver: _____

Printed name: _____

Date: _____

Appendix C
Laboratory methods

Determination of vitamins A (retinol) in human plasma or serum samples by high performance liquid chromatography (HPLC).

Department of Human Nutrition
University of Stellenbosch and
Tygerberg Hospital

27 March 1997
(revised March 2003)

Determination of vitamins A (retinol) in human plasma or serum samples.

1. Introduction

The test procedure is aimed at the quantitation of vitamin A levels in human plasma or serum samples. Normal levels for vitamin A range from 20 to 100µg/dL. Levels below these minima indicate progressive deficiency. In the case of vitamin A, this condition is important particularly in the case of pre-school children where it has major implications for their immune system.

2. Principle

Serum or plasma samples are first deproteinised by precipitation following which the fat-soluble components, including vitamin A, are extracted with hexane. After evaporation of the hexane, the residue is dissolved in methanol and aliquots used for quantitative determination by high performance liquid chromatography (HPLC). These operations are conducted in subdued light on account of the sensitivity of vitamin A to certain wavelengths of light.

3. Reference

The procedure is based on that described by L Catignani and J G Bieri: "Simultaneous determination of retinol and α-tocopherol in serum or plasma by liquid chromatography". Clinical Chemistry **29(4)** (1983) 708-712.

4. Materials

4.1 Reagents

Hexane and methanol (Burdick and Jackson, ACS/HPLC grade)

Ethanol (Merck, LiChrosolv grade)

Water (Distilled and purified with a Waters Milli-Q system)

Retinol (Sigma Chemical Co, Cat no R7632)

4.2 HPLC mobile phases

Solvent A: Add to HPLC-grade methanol, Milli-Q water (200mL) and make up to 2L. Mix thoroughly and degas by vacuum filtering through a filter disc (0.45µ).

Solvent B: HPLC-grade methanol.

5. Certified reference material

Fat-soluble serum vitamins (National Institute of Standards and Technology, Standard Reference Material 968c).

6. Equipment

HIMAC Centrifuge (Hitachi Model SCR20BA).

UV-Visible Spectrophotometer (Hitachi Model U-3200).

Waters HPLC system: two pumps (Model M45), autosampler (Model 717+) and automatic gradient controller (Model 680). Linear Programmable UV-VIS Detector (Model SSI 525).

Pentium 3 PC including 20Gb hard drive and 64Mb RAM.

EZChrom Elite/Client Server (ver 2.3) Chromatography Data System (Scientific Software Inc., USA) under Windows 95.

Spectra-Physics Recorder/Integrator (Model SP4290).

Supelco LC-18 HPLC column (250x4.6mm; 5µ beads).

Genie II vortex mixer

7. Standard solutions

7.1 Preparation of vitamin A standard solution

Because of the low concentration required for this standard solution it is necessary to use the following procedure (remember to work in subdued light):

Preparation of STOCK solution: Dissolve ± 12mg vitamin A in 10ml ethanol (HPLC grade). Store at approximately –20°C.

Purification of vitamin A: Aliquots from the STOCK solution are used to prepare a purified vitamin A solution of known concentration by the following procedure:

Prepare for an HPLC run using the following conditions:

- Chart speed 0.25cm/min
- Attenuation 128 and 1024

- Flow rate 1.5ml/min
- Mobile phase – Solvent A
- Detector wavelength 325nm.
- Supelcosil LC18 HPLC column (particle size of 5µm).

8. Precaution

All samples of human physiological fluids or tissue must be treated as potentially capable of transmitting disease and as such due precaution should be exercised by personnel when handling such samples.

9. Validation

9.1 The method is internationally published and accepted as correct.

9.2 The retinol (vitamin A) and α-tocopherol (vitamin E) used in the standard solutions are separated on the HPLC column in pure form (monitored at wavelength 292nm) for use as working solutions (see 7.1 and 7.2). The purity is gauged by the individual peaks on the chromatogram.

Note: Small spurious peaks (regarded as insignificant in the light of the proficiency test results) may elute close to the vitamin A and E peaks. These peaks are suspected to be isomeric forms but have not been identified as such due to lack of certified reference materials.

9.3 Proficiency testing has been on-going since 1996 on an annual basis. This international exercise is organized by the National Institute of Standards and Technology, Gaithersburg, Maryland, USA.

Determination of α_1-acid glycoprotein (AGP) in human plasma or serum

Department of Human Nutrition
University of Stellenbosch and
Tygerberg Hospital
Amended 3 April 2001

Introduction
The analytical procedure is aimed at the *in vitro* quantitative determination of α_1-acid glycoprotein (AGP) levels in human serum. Normal levels have been reported to be in the following ranges: Men, 0.5 – 1.3g/L and Women, 0.4 – 1.2g/L.

Principle
Samples are treated with the AGP reagent whose antibodies, when mixed with samples containing AGP, form immune complexes. These complexes are monitored by passing a beam of light through the complex suspension and the intensity of the resulting scattered light measured in the nephelometer is dependent upon the AGP content of the sample; by comparison to standards of known concentration, the AGP content of the sample can be determined. Precision control is assessed by running the control serum with each batch of samples assayed; the confidence intervals for controls are the assigned values (see AGP reagent kit insert with the protein control serum) ±15%.

References
L M Silverman,R H Christenson and G H Grant. Amino acids and Proteins, in "Textbook of Clinical Chemistry"; Ed. N W Tietz. Publ. W B Saunders, W Washington, USA, 1986, 591-592.

Reagents
Antiserum to human α_1-acid glycoprotein (OSAW).
Protein Standard Serum (OQIM)
Protein Control Serum (OQIO)
Reaction Buffer (OUMS)
All are obtained from Dade-Behring Marburg GmbH, Germany.
Saline (used for sample dilution).

Equipment
Nephelometer; Behring Model BN 100 Analyser coupled to a computer (Apple Macintosh LC II) loaded with Software Version N1.13A and an EPSON LX 400 dot-matrix printer.
Sample Cups (OVCM)
Cuvette Segments (OVCN)

Validation - Internal and external controls are used

Determination of C-reactive protein (CRP) in human plasma or serum

Department of Human Nutrition
University of Stellenbosch
Amended April 2002

Introduction

The analytical procedure is aimed at the *in vitro* quantitative determination of the acute phase protein, C-reactive protein (CRP) levels in human plasma or serum. Normal levels have been reported to have an upper limit of 5.0 mg/L.

Principle

Samples are treated with the CRP reagent consisting of polystyrene particles coated with antibodies to CRP which agglutinate when mixed with samples containing CRP. The agglutination is monitored and the intensity of the resulting scattered light in the nephelometer is dependent upon the CRP content of the sample so that, by comparison to standards of known concentration, the CRP content of the sample can be determined. Precision control is assessed by running the control serum with each batch of samples assayed; the coefficient of variation should be ≤5%.

References

M B Pepys. C-Reactive protein fifty years on. Lancet 1 (1981) 653-657.

C R H Kind and M B Pepys. The role of serum C-reactive protein (CRP) measurement in clinical practice. Int Med 5 (1984) 112-151.

S P Ballou and I Kushner: C-Reactive protein and the acute phase response. Adv Intern Med. 37 (1992) 313-336.

Reagents

CRP Reagent, CRP Standard and CRP Accelerator supplied as a CRP Reagent Kit (OUSV).
Reaction Buffer (OUMS)
CRP Control Serum (OUKU)
Diluent (OUMT)
All are obtained from Behring AG, Marburg, Germany.

Equipment

Nephelometer; Behring Model BN 100 Analyser coupled to computer (BN 100 terminal) loaded with Software Version N1.13A) and an EPSON LX 400 dot-matrix printer.

Sample Cups (OVCM)

Cuvette Segments (OVCN)

VALIDATION

Internal and external controls are used

Spectrophotometric determination of retinol in fortified sugar

From INCAP :November 2002

I. REFERENCES

Arroyave G. and Funes C. de (1974) Enriquecimiento de Azúcar con Vitamina A. Método para la Determinación Cuantitativa de Retinol en Azúcar Blanca de Mesa. *Arch. Latinoamer. Nutr.* **24**:147-153.

II. PRINCIPLE

This method is an adaptation of the method developed by Arroyave and Funes (1974). The procedure uses five to ten times less reagent volume than the original method, and its accuracy is similar. The precision, however, is somewhat lower, although highly satisfactory. The method requires the extraction of retinyl palmitate extraction in hexane. Retinol concentration is determined by its absorbance at 326 nm. This method does not usually require irradiation with UV light, because, the absorbance of the extract at 326 nm is essential only due to the retinol in sugar.

III. CRITICAL POINTS AND CAUTIONS

A spectrophotometer capable of reading 326 nm is essential. This is because the concentration of the retinol standards has to be verified by spectrophotometric analysis. Given the importance of the spectrophotometer for ensuring the accuracy and reliability of the retinol determinations, it should be calibrated frequently following the instructions provided by the manufacturer, especially to confirm the calibration of the monochromator. This confirmation should be carried out frequently and not only when a new lamp is installed.

Once retinyl palmitate has been extracted in hexane, the analysis should not be interrupted. Based on the experience at the INCAP laboratory, if the variability between replicates of the same solution is greater than 5 percent, the results should be rejected and the extractions repeated. The recovery of the method is at least 91%.

IV. EQUIPMENT

- UV Spectrophotometer (326 nm)
- Vortex mixer
- Water bath (50-60°C)

V. MATERIALS

- 100-150 mL beaker
- 20 mL test tubes with screw caps
- Aspiration bulbs for Pasteur pipettes and serologic pipettes
- Black clothing
- Glass rods
- Pasteur pipettes
- Spatulas
- Spectrophotometer cuvettes
- Test tube rack
- Volumetric flasks (100 mL)
- Volumetric pipettes (2, 3 mL)

VI. REAGENTS

- Absolute ethanol p.a., (C_2H_5OH), purity=99.8%, MW=46.07, d=0.79 g/mL, Art. Merck. 983
- Phenolphthalein ($C_{20}H_{14}O4$), MW=318.33, Art. Merck. 7233
- Hexane p.a. (C_6H_{14}), purity=99%, MW=86.18, d=0.66 g/mL
- Sodium hydroxide (NaOH), purity=97%, MW=40.00, Art. Merck. 6478

VII. PROCEDURE

1. Homogenize the sample within the bag with gentle rotary movements.

2. Weigh approximately 20 g of sugar, recording the exact weights to three decimal places and dissolve with 60-80 mL 0.1N NaOH in a 150-mL beaker. Use a glass rod to completely dissolve the sample.

3. Incubate in water bath at 50-60°C for 15 min. Cool at room temperature. Add 2-3 drops of 1% (w/v) phenolphthalein prepared in ethanol.

4. Transfer to a 100 mL volumetric flask. Rinse the beaker with small amounts of 0.1N NaOH and transfer the washings to the volumetric flask. Make up to 100 mL with 0.1 N NaOH and mix.

5. Measure 2 mL of the solution prepared in step 4, into three 20 mL test tubes. Prepare in triplicate a reagent blank with 0.1 N NaOH following the same procedure as for the samples.

6. Add 2 mL of absolute ethanol to each tube. Mix in the vortex mixer for 5 seconds.

7. Measure 3 mL of hexane and add it to each tube from step 5. Immediately close with a cap each tube and mix vigorously with the vortex mixer for 30 seconds to ensure complete extraction of the retinyl palmitate. Open the tubes briefly to release the vapour pressure. Allow separation of top organic solvent phase.

8. As soon as possible, transfer the organic phase, using a Pasteur pipette to a 1 cm light path spectrophotometer cuvette and read the absorbance at 326 nm. Adjust the zero of spectrophotometer with hexane before each reading. If UV-light spectrophotometer is not available, a visible light spectrophotometer may be use but the sensibility of the method decreases (Table 1).

VIII. CALCULATIONS

The retinyl palmitate concentration of the sugar sample is calculated using the following equation:

$$\text{Re}tinyl\ \ palmitate\ (mg/Kg) = \frac{Abs_{corrected}}{a}\ x\ \frac{Vh}{Vaz}\ x\ \frac{VI}{w}\ x\ \frac{CF_{spec}}{R}$$

Where:

$Abs_{corrected} = Abs_{sample} - Abs_{blank}$

And Abs_{blank} is the average for the three readings, which should be less than 0.050

The equation parameters are:

PARAMETER	EXPLANATION	VALUE
A	Retinyl palmitate absorption coefficient in hexane (mg^{-1} cm^{-1} mL).....	0.092
Vh	Volume of the organic phase (mL)	3.0
Vaz	Volume of the aliquot analyzed from the sugar solution (mL)	2.0
VI	Volume of the initial solution of the sample (mL).....	100.0
W	Weight of the sample (g).....	data from weight
R	Recovery	0.905
CF_{spec}	Correction factor of the spectrophotometer. Ideally	?

To express the results as unesterified retinol, the ratio of the molecular weights of retinol/retinyl palmitate (286.46/524.84 = 0.546), must be taken into consideration.

IX: VARIATIONS

For analysis of refined fortified sugar with vitamin A, follow the next steps to prepare the sample solution:

1. Weigh approximately 100 g of sugar, recording the exact weights to three decimal places and dissolve with 100 mL distilled hot water (about 80°C) in a 150-mL beaker. Use a glass rod to completely dissolve the sample.

2. Let the solution reach room temperature and transfer the solution quantitatively to a 200-mL volumetric flask. Wash the beaker with distilled water and transfer the washings to the flask. Agitate the solution thoroughly and make up to volume with distilled water.

3. In a 20-mL test tube, place 2-mL sample solution and add 2 drops 0.1-N sodium hydroxide and agitate for 5 seconds.

4. Add 2 drops 1%(w/v) phenolphthalein and 3 mL absolute ethanol. Agitate in a vortex mixer for 5 seconds.

5. Add 3 mL hexane and agitate vigorously in a vortex mixer for 30 seconds.

6. Let the phases separate. If the phases does not separate, add a few drops of absolute ethanol.

7. Read the absorbance of the hexane phase in a UV-spectrophotometer at 326 nm.

Table 1

CORRECTION FACTORS FOR RETINOL ABSORBANCE AT DIFFERENT WAVELENGTHS USIN A SOURCE OF VISIBLE LIGHT

WAVELENGTH (nm)	CORRECTION FACTOR
325 (visible light)	1.007
330	1.066
335	1.117
340	1.360
345	1.622
350	2.030

Verfification or the efficiency of the extraction

To verify the efficiency of the extraction, a recovery assay should be done using the following suggested procedures[1]:

1. Using a sample with unfortified sugar follows steps G.1-4 of the analytical procedure. At this point, add to two of three test tubes 3 mL of absolute ethanol and then 1 mL of an ethanol solution of retinol palmitate of a known concentration (approximately 20 micrograms of retinol palmitate per milliliter[2]), that is the control. To the third tube, that is, the blank, add 4 mL of absolute ethanol. Continue with the analytical procedure from the step G.6. Read the absorbance of the retinyl palmitate controls and the blank. Subtract absorbance of the blank from the mean absorbance of the controls. This is the absorbance due to the retinyl palmitate added (*s*)

2. Independently prepare a retinol palmitate solution as follows. Measure 1 mL of the same retinyl palmitate solution that was used above into 5 mL volumetric flask. Make up the volume with ethanol. Read the absorbance of this solution and multiply by 0.98 to

[1] This assay uses pure retinyl palmitate rather than retinyl palmitate beadlets; however, in the experience of INCAP, the results are practically the same.

[2] Prepare from a primary solution of 100 µg/mL of retinyl palmitate in ethanol; keep at –20°C in a nitrogen atmosphere and a dark container. The 20-µg/mL solution should have an absorbance of near 2.0 at 325 nm against absolute ethanol.

compensate for the higher of retinyl palmitate in ethanol than hexane. This product (t) is the theoretical absorbance that would have been found if recovery efficiency was 100 percent.

3. Calculate recovery (R) as follows:

$$R = \frac{s}{t} x\ 100$$

Determination of retinol in fortified sugar using spectrophotometer and CRAFTi portable fluorimeter - Round 1 July

Procedure

1. Homogenized the sample with gentle rotary movements.

2. Weighed approximately 20g of sugar, recording the exact weights to three decimal places and dissolved with 60 – 80ml 0.1N Na0H in a 150ml beaker. Used glass rod to completely dissolve the sample.

3. Incubated in water bath at 50-60 ^{o}C for 15min. Cooled at room temperature.

4. Transferred to a 100ml volumetric flask. Rinsed the beaker with small amounts to 0.1N Na0H and transferred the washings to the volumetric flask. Made up to 100ml with 0.1N Na0H and mixed.

5. Measured 2ml of solution prepared above, into two 20ml test tubes. Prepared in duplicate a reagent blank with 0.1N 0H following the same procedure as for the samples.

6. Added 2ml of absolute ethanol to each tube. Mixed in the vortex mixer for 5 seconds.

7. Measured 3ml of hexane and added it to each tube. Covered the tubes with aluminium foil and mixed vigorously with the vortex mixer for 30 seconds to ensure complete extraction of retinyl palmitate. Allowed separation of top organic solvent phase to take place.

8. Transferred the organic phase, using a Pasteur pipette to a icon light path spectrophotometer curvette and read the absorbance at 325nm. Before taking the readings the UV spectrophotometer was zeroed.

9. After reading in the UV spectrophotometer, the same organic phase was transferred into the crafti portable fluorimeter curvette and readings were taken.

   Before taking down readings the instrument was calibrated according to instructions in the manual. (Portable Fluorimeter for measuring vitamin A concentrations in fortified foods: Manual revision dated 24th June 2003).

CALCULATIONS

UV Spectrophotometer

$$\text{Retinyl palmitate (mg/kg)} = \text{Abs corrected} \times \frac{983.67}{W}$$

Craft Portable Fluorimeter

Retinal Palmitate (µg/ml) = Average reading – blank) x 1.83

= Convert answer to µg/ml or mg/kg

MATERIALS

1. 100 – 150ml beakers.
2. 20ml test tubes.

3. Aspiration bulbs for Pasteur pipettes and serological pipettes
4. Black clothing.
5. Glass rods.
6. Pasteur pipettes.
7. Spatulas.
8. Spectrophotometer curvettes.
9. Fluorimeter curvettes.
10. Test tube rack.
11. Volumetric flasks (100ml).
12. Volumetric pipettes (2,3ml).

REAGENTS

1. Absolute ethanol (purity 99.8%).
2. Hexane (redistilled)
3. Sodium Hydroxide (Na0H)

EQUIPMENT

1. UV Spectrophotometer.
2. Crafti portable fluorimeter.
3. Vortex mixer.
4. Water bath (50 – 60 ^{o}C).

Determination of retinol in fortified sugar using spectrophotometer and Crafti portable fluorimeter – modified for round 2 November

Procedure

1. Homogenized the sample with gentle rotary movements.

2. Weighed approximately 20g of sugar, recording the exact weights to three decimal places and dissolved with 60 – 80ml 0.1N Na0H in a 150ml beaker. Used glass rod to completely dissolve the sample.

3. Incubated in water bath at 50-60 ^{o}C for 15min. Cooled at room temperature.

4. Transferred to a 100ml volumetric flask. Rinsed the beaker with small amounts to 0.1N Na0H and transferred the washings to the volumetric flask. Made up to 100ml with 0.1N Na0H and mixed.

5. Measured 2ml of solution prepared above, into two 20ml test tubes. Prepared in duplicate a reagent blank with 0.1N 0H following the same procedure as for the samples.

6. Added 2ml of absolute ethanol to each tube. Mixed in the vortex mixer for 5 seconds.

7. Measured3ml of hexane and added it to each tube. Covered the tubes with aluminium foil and mixed vigorously with the vortex mixer for 30 seconds to ensure complete extraction of retinyl palmitate. Allowed separation of top organic solvent phase to take place.

8. Transferred the organic phase, using a Pasteur pipette to a icon light path spectrophotometer curvette and read the absorbance at 325nm. Before taking the readings the UV spectrophotometer was zeroed.

9. After reading in the UV spectrophotometer, the same organic phase was transferred into the crafti portable fluorimeter curvette and readings were taken.

   Before taking down readings the instrument was calibrated according to instructions in the manual. (Portable Fluorimeter for measuring vitamin A concentrations in fortified foods: Manual revision dated 24th June 2003).

Calculations
UV Spectrophotometer

$$\textit{Retinyl palmitate (mg/kg)} = \textit{Abs corrected} \times \frac{983.67}{W}$$

Craft Portable Fluorimeter

Retinal Palmitate (ug/ml) = *Average reading – blank) x 1.83*
= *Convert answer to ug/ml*
ug/ml = mg/kg

Appendix D

Map reading

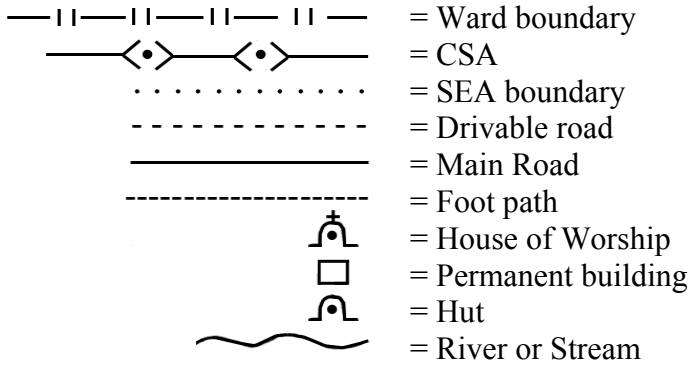
Map reading

Zambia is divided into nine provinces. In turn, each province is subdivided into districts, each district into constituencies, and each constituency into wards. In addition to these administrative units, each ward is divided into convenient areas called Census Supervisory Areas (CSAs), and in turn each CSA is divided into Standard Enumeration Areas (SEAs). In total Zambia has 72 districts, 150 constituencies, 1,289 wards, about 4,400 CSAs and about 16,400 SEAs (ZDHS, 2001).

SEAs in rural areas have between 300-600 households while these in urban areas have between 600-800 households. On average a CSA has 3 SEAs. The key below shows the major boundary and structure definitions used during map reading.

Note: SEA = Cluster

Key: Main and Magnified Maps.



!:10,000 urban scale on map
1:50,000 rural scale on map

i.e. 1 unit on map = 50, 000 units of road
1 unit = 1 metre

Map heading:

CSO/ 3 – 303/ 041 – 14/ 1 – 02 where:

CSO/3 – 3 = province
03 = district
041 = constituency
14 = ward
1 = rural
02 = CSA

Appendix E

Field methods

The Finger Puncture Procedure

A finger puncture procedure will be conducted for survey participants i.e. randomly selected child between 6 - 59 months and mother/female caregiver 15 - 45 years. Blood collected via a finger puncture will be used to prepare a *malaria thick smear*, to measure a *hemoglobin* level using a *cuvette* and a *Hemocue instrument*, and to collect *250-500 µl of blood into a Microtainer for vitamin A and acute phase protein analysis*. In order to obtain enough blood for all of these purposes, it is essential that a **good deep stick is done which will give a good flow of blood.** Remember, each subject can only be punctured once so there will be **only one opportunity to collect all the necessary blood.**

1. Identify the selected survey participants. For this study there will be 2 participants identified per household: a child and mother/caregiver. The survey team members administering the questionnaire will identify the participants.

2. Check the ID on the labels for the blood collection match the ones stuck on the questionnaire. Have the labels ready to put onto the microtainers once the blood has been collected and label the malaria slide in advance. Be very careful not confuse the ID #s or samples with each other.

3. Prepare your collection equipment using one "Household kit", which contains the following supplies: 4 Lancets (**2 Tenderlett-red for adults and 2 Tenderlett Junior-blue, for children under 2 years of age**), 4 alcohol pads, 4 gauze, 2 band-aids, 3 microscope slides, labels, 3 Microtainers, 1 blue absorbent pad, 4 pairs of gloves, 3 hemocue cuvettes, kim wipes and safety glasses (see table of daily supplies).

4. Label malaria slides; put the label over the frosted end of the slide (if there is a frosted end). Label microtainers correctly after blood collection (putting label on after blood collection allows the blood volume to be seen during the collection). Wrap the label around the microtainer tube.

5. **Be sure you are wearing your gloves and safety glasses before you begin**. With each participant, select the skin puncture site on the side of the finger (not too close to the nail bed).You may use either hand, but the less dominant hand is usually not as calloused. Use the middle or ring finger (3^{rd} or 4^{th} finger). Do not use the last finger (5^{th} finger) or the thumb.

6. Thoroughly clean the puncture site with a 70% isopropyl alcohol pad (continue to clean the site until no dirt appears on the alcohol pad). Wipe the site dry with gauze. (Residual alcohol may dilute sample).

7. Puncture the site with a disposable lancet. Hold the finger firmly to immobilize the finger, as some patient's response is to pull away as you perform the skin puncture. Use moderate pressure and depress the plunger completely, then release the plunger and remove the lancet. Discard the lancet in the puncture-resistant sharps container.

8. Make puncture and collect first large drop of blood onto a properly labeled malaria microscope slide (see more detailed explanation later). It is extremely important to get a good deep stick the first time so there will be plenty of blood to collect. If the first finger stick is not good, then do not waste time trying to get the blood. Ask permission from the

mother to do another finger stick. If appropriate, make sure the blood is on the same side of the slide that has the rough frosted end and label. Place the slide in the sun at the pre-selected safe location. A team member may be able to assist you with this while you complete the blood collection.

9. Collect the blood in the microtainer tube by applying light pressure opposite the puncture site until a drop appears. Use the microtainer as a scoop to guide the drop into the tube. Do not scrape the side of the finger to collect the blood. Continue to squeeze the finger and the whole hand to help blood flow. You may use the gauze to wipe away some of the blood that covers the finger before applying more pressure**.** Continue to collect blood droplets until you reach the 500 µL fill line (a minimum of 250µL is required to obtain accurate results). Do not overfill. Mix the vial by flicking it with you finger during the collection to prevent micro-clots from forming.

10. Replace the microtainer cap and **gently** invert the tube 8 to 10 times to ensure adequate mixing of the blood with the anti-coagulant in the tubes. Microclotting is the biggest problem with microtainer vials and anything you can do to speed up the collection of the blood into the vial the better.

11. Using gauze, place gentle pressure on the site to stop bleeding. Apply a band-aid.

12. Fill the hemacue cuvette by introducing the pointed tip of the cuvette into the middle of a drop of blood in the microtainer. The cuvette should be allowed to fill by capillary action in one continuous motion (*do not "top off" the cuvette*). The cuvette must be completely filled (without any large air bubbles) or discarded and the test repeated from another drop of blood.

13. Wipe off any excess blood from the outside of the cuvette using a Kim wipe, being careful not to touch the outer curved edge; use an action like wiping a butter knife onto bread. This will assure that no blood is "sucked out" of the cuvette when wiping it.

14. Place the filled cuvette in the Hemocue instrument holder right away and gently insert slide arm to the "Measuring" position. The results will be displayed in approximately 15-45 seconds and will remain displayed for 4 minutes or until the slide arm is pulled out for removal of the cuvette.

15. Record the results from the hemacue, dispose of the cuvette and lancet in the sharps container, dispose all other materials in the biohazard bag. Turn off the Hemocue instrument. Double check that everything is labeled correctly. Place the microtainer in the cooler and prepare to move to the next household.

16. Store the blood in gridded boxes with lid and place into a cold box with frozen cold packs. Make sure cold packs are frozen and some type of packing material (newspaper) is between the specimens and the cold packs. To keep specimens cool, insulation material like newspaper may be placed on top of the box of specimens and two more cold packs placed on top. The whole blood specimens should remain cool but should not freeze.

17. At the hospital/clinic/lab, the samples should be arranged in numerical order so that it will be easy to keep track of your samples.

18. Spin down the blood in the microtainers to obtain PLASMA (not serum). The

centrifuge should spin at 1000-1300 relative centrifugal force (RCF) (g force) for 10 minutes. This is usually around 2,800 revolutions per minute (RPMs) for an average size centrifuge but you can measure the radius and calculate the g force. To save time, use multiple centrifuges if available.

19. While one batch is spinning, process the batch that has already been centrifuged.

20. Label empty 500 µl plastic cryovials with the matching coded ID label for the specimen collected.

21. Once the centrifugation of a batch is complete, CAREFULLY take off the plasma with a plastic or glass Pasteur pipette. DO NOT PULL UP THE RED CELLS OR PLATELETS into the plasma sample. If you have never done this before, practice before collection of actual samples. If red blood cells are accidentally pipetted into the plasma sample, put the plasma and red cell mixture back into the microtainer and re-spin.

22. Obtain as much of the plasma as possible, being very careful not to introduce red cells into the plasma vial. Screw the cap on very tightly onto the cryovial and immediately place into a gridded box, in numerical order and place in a freezer in an upright position. Once frozen the vials should be kept frozen.

23. Take all plasma samples to Dr Kankasa's lab at University Teaching Hospital, Lusaka, keeping them in cold and dark conditions at all times. Keep a record or list of the plasma vials.

24. Plasma samples should be shipped on dry ice to the HPLC lab in South Africa for analysis. Keep a record or list of the vials sent off and also send another copy of the list along with the samples, using a form called a Sample Shipping manifest form.

Helpful suggestions for the finger stick:

If hands are cold it is helpful if the subject rubs hands together, mother can help warm child's hands, prior to testing to stimulate blood flow to the capillaries.

Avoid squeezing with thumb and forefinger in a V pattern around the puncture site as this will decrease the blood flow.

When applying pressure to stimulate flow it is helpful to apply pressure and then relax pressure momentarily to allow blood to flow into the capillary bed.

While performing puncture have hand of patient below their heart level. If the arm is above the heart it slows the blood flow to the hand.

Hold survey participant's hand in a downward fashion to allow gravity to assist with blood droplet formation.

When doing a finger-stick on young children it usually is easier to grasp all of their fingers together with your entire hand and apply pressure to all four fingers than to work with one small finger.

Important facts for the HemoCue Hemoglobin testing:

Store Hemocue cuvettes at room temperature. Do not allow storage temperature to exceed 86 degrees F.

The red control cuvette should be used to check the instrument each morning and if it gives a result within the range on the card, go on to check the Hemacue with liquid QC (low and normal). Record results. Repeat this procedure at the beginning of each day and at the end of the day. In addition use red control cuvette at regular intervals during the day to make sure the Hemacue is still giving a result in the correct range. If machine is dropped or anything unusual happens to it please carry out a check with the red cuvette.

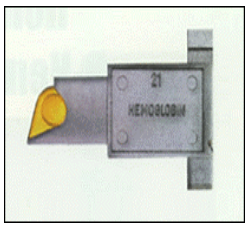
Tightly reseal round red top container after removing only the number of cuvettes to be used for immediate testing.

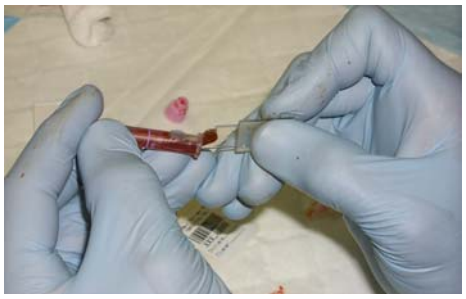
After filling the cuvette and wipe off excess blood, place the cuvette into the slide arm and **gently** slide the arm into the instrument. Never SLAM the slide arm.

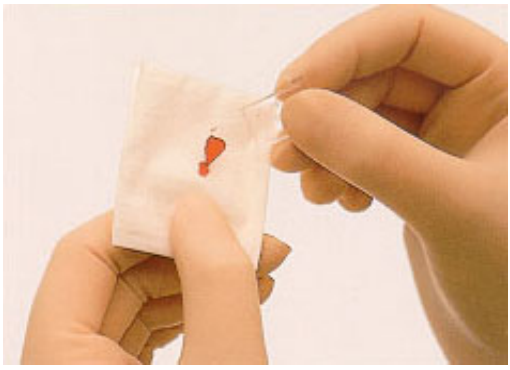
Read the cuvette within a maximum of 5 minutes at the most and record the results.

Clean the back slide arm daily with alcohol or mild soap solution. Make sure it is dry before returning to the instrument. Check Hemacue for contamination throughout the day and clean if any blood is spilled.

The Hemocue™ Procedure
Zambia, November 2003







USING THE HEMOCUE™

Anemia, as determined by low hemoglobin (Hb), is often used as a proxy indicator for iron deficiency. One instrument used in testing for anemia is a photometer called HemoCue™ which tests the Hb concentration using a single drop of blood. This is a robust instrument that can give accurate readings in a field setting. However, errors in Hb assessment occur if appropriate procedures and techniques are not followed. Use of inappropriate procedures/techniques may lead to wide variations in Hb values. This then leads to erroneous estimates of anemia prevalence in the population tested.

The following steps are recommended to help ensure reliable testing of Hb using the HemoCue™ photometer.

Using the HemoCue™ for Hemoglobin Testing
Daily
At the beginning of each survey day, check the instrument accuracy using the control cuvette and liquid controls. If readings are in question, clean the cuvette holder and control cuvette with a dry wipe. If readings continue to be outside the correct range, do not use the instrument. It should be serviced or replaced.

Common Problems to Avoid

1) Keep the instrument clean, especially the cuvette holder.

A swab (Q-tip) dabbed with alcohol can be used to clean away any dirt or dried blood. This should be done at least once a day or when there is a visible build-up of dirt or blood. Be sure the cuvette holder is dry before re-inserting it in the machine.

2) Ensure instrument accuracy

Check the accuracy of the instrument at least daily, or when performance is questioned, using the control cuvette which comes with each Hemocue™ instrument. Keep a daily log of accuracy readings. If the accuracy readings are outside the range of the control cuvette, and the Hemocue™ is clean, then the instrument needs to be replaced.

3) Keep cuvettes clean, dry and away from heat

Unsealed containers of cuvettes can be kept for 3 months after opening, and then they should be discarded. Keep the container lid closed when not being used to avoid unnecessary exposure of the cuvettes to air, especially in humid conditions. Heat and moisture will denature the chemicals in the cuvette which can lead to inaccurate Hb measurements.

4) **Make sure the finger stick is adequate**

Wide variations can occur in Hb measurements if the finger stick is inadequate (basically equated to the finger stick not being deep enough to allow adequate flow of blood and a representative concentration of red blood cells). In most cases if the finger stick is done poorly, Hb values will be underestimated and the prevalence of anemia will be overestimated.

5) Avoid poor technique

- **Milking the finger** (usually related to an inadequate finger stick) to obtain proper blood flow which will underestimate Hb readings.

- **Mixing alcohol with the blood.** The patient's finger should be totally dried before the finger prick is performed. Use alcohol to clean the finger before the prick is made and then wipe with a dry wipe to avoid any mixing of the blood with alcohol. Wiping away the first 2 drops of blood also will minimize the mixing of sweat with blood in hot, humid climates. This error usually underestimates the Hb reading.

  Avoid removing a cuvette from its container when your fingers are wet with alcohol. Alcohol coming in contact with the cuvette can denature the needed chemical in the cuvette selected, as well as, other cuvettes still in the container.

- **Obstructing blood flow to the puncture site.** Do not hold the subject's hand so tightly as to obstruct blood flow to the fingers.

6) Adequately fill the cuvette

The cuvette needs to be filled with a drop of blood **in one continuous motion.** Do not "top off" the cuvette that is not completely filled. This results in erroneous Hb readings...usually too high.

Any signs of air-bubbles mean that the cuvette has not been filled adequately and should be discarded and a new cuvette used. The presence of bubbles will usually underestimate the Hb reading.

7) Do not "slam" the cuvette holder into position for reading.
This will avoid spraying blood droplets which can contaminate the scanner.

Summary of common problems and solutions related to capillary sampling and use of the HemoCue photometer.

PROBLEM	SOLUTION
Not preparing all needed materials before testing a subject.	Place cuvette, alcohol swab, gauze pad, and lancet on work surface; turn on photometer; pull out the cuvette holder to "locked" position so that digital screen reads "READY"; put on latex gloves.
Selecting a cuvette from its jar with fingers wet with alcohol (the alcohol denatures the chemicals inside the cuvette; thus, the selected cuvette as well others inside the jar can be denatured).	Take cuvette out of its container before handling a wet alcohol swab.
Not drying finger completely after disinfecting with alcohol (since the HemoCue cuvette only hold 10 µL of blood, its volume can be easily affected by even a trace of alcohol on the puncture site).	Firmly wipe the finger using a dry gauze pad. Firm wiping can also help stimulate blood flow to the finger tip.
Inappropriate and shallow finger puncture.	An appropriately deep puncture done with a "quick stab" will result in a better blood flow and more rapid completion of the test. (A number of brands of high quality single-use lancets are now available in the market).
Restricting blood flow to finger tip following the finger-stick.	Release the subject's finger after the stick to allow blood flow; also hold the subject's hand without squeezing and restricting blood flow to the finger tip.
Milking the finger (this will lead to mixing of interstitial fluids with the blood drop leading to an inaccurate Hb reading...usually too low).	A good finger-stick should result in spontaneous blood negating the need to apply pressure to the finger. If stimulating blood flow is needed, apply **gentle** pressure with the thumb on the opposite side of the finger from the puncture site.
Holding cuvette in inverted position (slit facing down) during filling (this can lead to air bubbles being trapped resulting in erroneous result).	Hold the cuvette with the slit facing up and the pointed tip touching the blood drop.
"Topping off" a partially filled cuvette with repeated blood collection (the reagents in the cuvette are denatured upon contact with the initial amount of blood; red cells of blood introduced later will not be adequately analyzed).	Allow a large blood drop to form on the finger so that it will completely fill the cuvette in **one motion**. Once filled, hold the cuvette in place for about 2-3 seconds longer to ensure complete filling.
Not cleaning off blood on outside of cuvette before testing (can result in erroneously high Hb reading).	Wipe off excess blood from sides of cuvette using a "butter knife" motion to ensure that blood from inside the cuvette is not removed.
"Slamming" the cuvette holder into place (can lead to blood drops spattering inside the reading chamber).	Push the cuvette holder gently into position. Once or twice a day clean the cuvette holder with alcohol swab and completely dry before testing. Periodically clean the reading chamber with dry gauze.

Zambia Vitamin A Survey Hb QC Form

November 2003

Date: _____/____/_____ (Month) (Day) (Year)

Place: _____

Analyst: _____

HemoCue Serial # _____

Cuvette Lot # _____

Control Vial Lot #s: Low: _____

Normal: _____

Quality control for blood hemoglobin results (grams per deciliter - - - - g/dL)

		Morning			Evening		
Date	***Calibration Slide Specific For Each Instrument***	**Low Target:**	**Normal Target:**	**Comments**	**Low Target:**	**Normal Target:**	**Comments**

Additional Comments: _____

_____

Hemocue Photometer Cleaning Instructions MK II

Materials needed: Q-tips and cold water

I. Black slide holder

A. Remove the black slide holder and clean with a mild detergent and water or swab with alcohol.

B. Rinse thoroughly with water and dry completely.

II. **Optronic unit**

Turn off photometer

A. Moisten a Q-tip with cold water and squeeze out any excess water.

B. With the black slide holder removed, insert the Q-tip approximately1 – 1½ inches into the black slide holder opening. **Apply pressure upwards as you insert the Q-tip,** you should feel a slight ridge or indentation. This is the sensor window

C. Clean a 1 inch square area around the ridge. Repeat with clean damp Q-tips until no residue is observed on the Q-tip. Repeat procedure with clean damp Q-tip pressing down, however you will not feel ridge.

D. With a **dry** Q-tip, swab the same area just cleaned. Hold instrument up with slide holder opening pointed down and tap on the bottom of the Hemacue, this will allow any dried blood to be removed, wait 5 minutes to allow to dry, before turning the power to instrument on again.

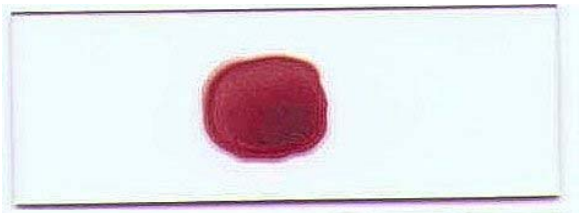
E. Check the photometer using the control cuvette or liquid controls.

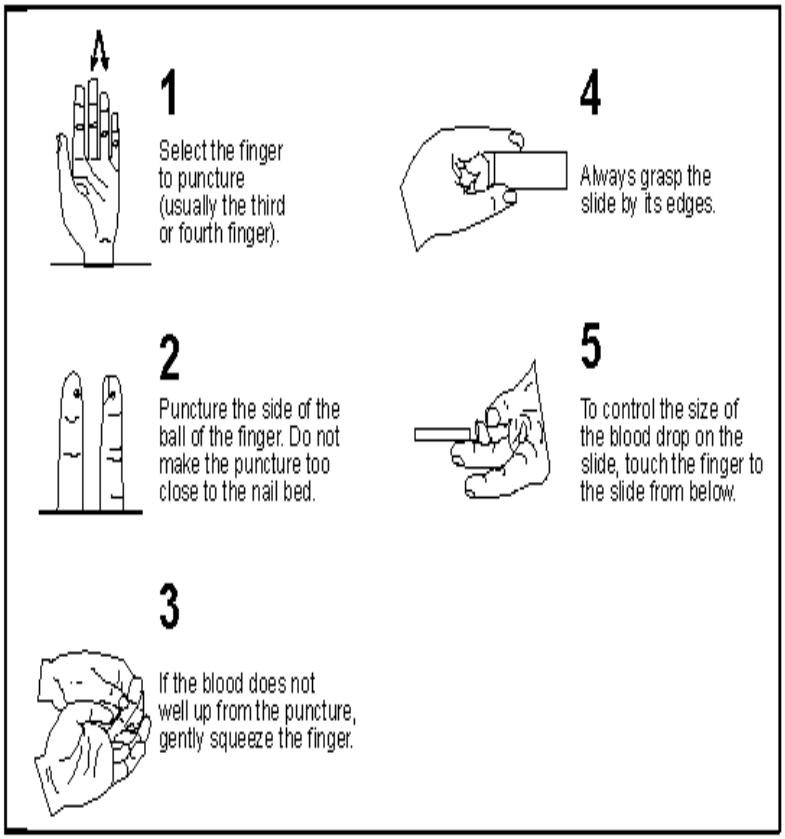
F. It may be necessary to clean the control cuvette. Hold mirrored area of the control cuvette under running warm water for a few seconds. Polish the area very gently using a gauze or Q-tip to remove all water. Polish one side and then the other to avoid putting to much pressure on the mirrored area and **be gentle,** so that the delicate cuvette does not crack.

The Malaria Thick Smear

Slide Preparation Procedure

1. Place the correct label on the rough frosted end of the slide.
2. Conduct the finger stick according to the finger puncture procedure.
3. Using the first drop of blood, touch the clean, labeled microscope slide near one end to the formed blood drop. (**Make sure that blood drop is placed on same side of the slide that the label is on**).
4. Spread the drop of blood with the corner of another slide to make an area about 1 cm in diameter.
5. Correct thickness is attained when newsprint is barely legible through the smear.





Appendix F

Table of Design Effects

Prevalence, 95% confidence intervals (CI), and design effects (DEFF) for key indicators for children aged 6 – 59 months.

Prevalence	Survey – July 2003				Survey – November 2003				July and November data combined			
	n	Prevalence %	95 %CI	DEFF	n	Prevalence %	95% CI	DEFF	n	Prevalence %	95% CI	DEFF
Severe VAD (Plasma retinol ≤ 0.35 µmol/L)	317	5.8	2.4, 9.2	1.6	342	4.3	1.9, 6.6	1.1	659	5.0	2.8, 7.2	1.7
VAD (Plasma retinol ≤0.7 µmol/L	317	53.3	44.3, 62.4	2.5	342	54.7	45.3, 64.1	2.9	659	54.1	46.5, 61.6	3.6
Anaemia (Haemoglobin < 110 g/L)	353	59.3	51.5, 67.0	2.1	371	47.0	39.2, 54.8	2.1	724	52.9	46.7, 59.2	2.7
CRP (≥5 g/L)	119	45.4	34.1, 56.7	1.4	178	35.6	26.4, 44.7	1.5	297	39.4	31.5, 47.4	1.9
Cough	380	69.1	63.3, 74.8	1.4	386	58.7	53.2, 64.2	1.1	766	63.9	60.1, 67.6	1.1
Diarrhoea	378	29.7	22.9, 36.5	2.0	385	34.7	28.1, 41.3	1.8	763	32.2	26.8, 37.6	2.5
Fever	377	50.8	42.4, 59.1	2.5	386	42.5	37.0, 47.9	1.1	763	46.6	41.0, 52.2	2.3
Malaria parasitaemia	367	40.1	27.9, 52.3	5.4	373	21.4	12.3, 30.6	4.4	744	30.7	20.6, 40.8	8.4
Vitamin A supplement (June)	376	89.8	85.9, 93.7	1.5	376	84.8	80.5, 89.0	1.3	752	87.3	83.8, 90.8	2.0
Prevalence of adequately fortified sugar found at household (HH) and retail												
Fortified sugar (≥ 10 mg VA/kg sugar) in household	174	16.1	10.1, 21.7	1.0	127	19.7	11.0, 28.3	1.4	301	17.6	12.3, 22.9	1.4
Retail sugar (≥10 mg VA/kg sugar)	23	4.3	0, 15.5	0.7	31	12.9	0.1, 25.8	0.9	54	9.3	1.5, 17.0	0.8

Table showing prevalence, 95% confidence intervals (CI) and design effects (DEFF) for key parameters for non-pregnant women 15 – 49 years

Prevalence	July and November data combined			
	N	Prevalence %	95% CI	DEFF
VAD (Plasma retinol ≤ 0.7 µmol/L	583	13.4	9.4, 17.4	1.9
Anaemia (Haemoglobin < 120 g/L)	623	29.1	24.7, 33.4	1.4
CRP (≥ 5 g/L)	256	22.7	17.0, 28.3	1.1
Malaria parasitaemia	622	7.3	4.1, 10.3	2.1

Appendix G

Survey staff job descriptions

Supervisor Job Description

Qualifications
Experienced in household survey sampling and data collection.

Key Supervisor Responsibilities

1. Supervisor has authority to make day-to-day decisions for the team.
2. Liaison with local departments of health and arrange for community health workers as needed.
3. Ensure the safe and adequate collection of a blood sample to assess retinol (vitamin A) levels—assist with the process as needed.
4. Ensure proper training and preparedness of team members.
5. Ensure necessary supplies and equipment are available for use by survey team.
6. Review questionnaires to ensure they are properly completed and labeled at the household.
7. Monitor to ensure the proper labeling, safe storage and transport of blood samples and safe disposal of biohazardous materials.
8. Ensure retails sugar samples are collected from each cluster.
9. Serve as a resource for all questions and concerns of survey respondents and team members.
10. Maintain petty cash for fuel and miscellaneous expenses. Retain all receipts for fuel, sugar and all other purchases.
11. Assure all team members are working together to complete all daily tasks, regardless of specified job description.

Day-to Day Responsibilities

- Maintain supply of quality control materials between 0 to 8 degrees C.
- Ensuring availability of back-up HemoCue and extra supplies for all data and sample collection procedures.
- Review the daily plan with team members.
- Attach label sheet to the questionnaire.
- Assist teams to identify households pre-selected for the survey.
- Oversee the completion of questionnaire.
- Assist with blood sample collection as needed to ensure adequate sample.
- Ensure safe disposal of biohazardous materials.
- Review questionnaires upon completion of interviews for accuracy and completeness before leaving the cluster.
- Introduce team to local DHMT and facilitate their acceptance into the community.
- Oversee community health workers activities and payment for services (to be paid from lunch allowance).

NOTE:

1. ***Interviews may be performed by the nutritionist, lab technician, nurse or supervisor according to the needs of the team in the field.***
2. ***All team members are expected to assist one another as needed to complete all tasks in an efficient and timely manner. Only qualified professionals may draw blood or handle sample processing.***

***Interviewer Job Description**

Qualifications
Experienced nutritionists and laboratory technicians capable of effectively completing a detailed interview with respondents.

Interviewer Responsibilities

1. To introduce the survey and survey team to the household and obtain informed consent
2. To ensure that the survey questionnaires are completed accurately and fully
3. To collect sugar and salt samples from each household
4. To review questionnaires for completeness prior to leaving the household

Day-to-Day Responsibilities

1. Follow the directions of the team supervisor who will have given each interviewer a plan for the day. The supervisor will give each interviewer the name of the heads of each household and the assigned household number for the houses they will visit.

2. Complete the top of the household form and attach label to questionnaire before entering the household.

Each interviewer will be provided with a list of households to approach regarding participation in the survey. A questionnaire must be completed for each household that includes at least one child between the ages of 6 and 59 months and where informed consent is obtained. A total of 13 households must be completed in each cluster.

Interviewer supplies (nutritionists- enough to complete 7 households daily and lab technician enough to complete 6 households daily)

- Backpack
- Questionnaire
- Bag and tie to collect sugar samples
- Permanent marker (Sharpie) to write household number on the bag with the sugar
- Clipboard
- Pencil and eraser
- Pen
- Standard measuring spoons and cups.

NOTE:

1. **** Interviews may be performed by the nutritionist, lab technician, nurse or supervisor according to the needs of the team in the field.***

2. ***All team members are expected to assist one another as needed to complete all tasks in an efficient and timely manner. Only qualified professionals may draw blood or handle sample processing.***

Lab Technician Job Description

Qualifications
Trained laboratory technician with at least 3 years experience.

Key Supervisor Responsibilities
1. Ensure proper processing, storage and transport of blood samples.
2. Handover samples to handover to Dr Kankasa's Research Assistant at University Teaching Hospital, upon arrival in Lusaka

Day-to Day Responsibilities
1. Receive blood samples from the nurses at the end of the day
2. Identify lab facility where blood processing can be done, if available or appropriate alternative
3. Set up equipment and spin blood in centrifuge to separate plasma
4. Remove plasma and place in cryovial
5. Label cryovial and store as follows:
    - If freezing CAN be maintained until arrival in Lusaka- place at -20 º C as soon as possible.
    - If freezing CANNOT be maintained, store in refrigerator or cold box at 0 - 8º C until then.
6. Pack safely for transport to Lusaka (see instructions earlier) and handover to Dr Kankasa's Research Assistant at University Teaching Hospital, Lusaka upon arrival.

NOTE:

1. ***Interviews may be performed by the nutritionist, lab technician, nurse or supervisor according to the needs of the team in the field.***
2. ***All team members are expected to assist one another as needed to complete all tasks in an efficient and timely manner. Only qualified professionals may draw blood or handle sample processing.***

Nurse Job Description

Qualifications

Trained nurse with experience in drawing and labeling blood samples. Experience doing capillary blood draws preferred.

Nursing Responsibilities

1. Safe and effective collection of capillary blood from all survey participants
2. Correct labeling of all blood samples and documenting of results of hemoglobin
3. Ensure availability and safekeeping of equipment for drawing of blood at the household
4. Safe use and disposal of all equipment and supplies—maintain blood safety precautions
5. Prepare malaria slide, collect microtainer blood sample and test blood for hemoglobin in Hemocue.

Step by Step Blood Collection Process

1. Advance Preparation

   Ensure availability of proper functioning equipment and supplies prior to arriving at household

   Prepare household supply kits (See Daily Supply List)

2. Set-up at Household

   Introduce yourself at household

   Set up equipment and supplies

   Follow universal blood safety precautions at all times

   Complete quality control check (with standard cuvette and liquid controls) at first household of the day and record results

   Quality check with standard cuvette at mid-day

3. Collect Blood (See finger stick instructions)

4. Label Blood Samples

   Pre-printed label applied to: - Malaria slide; - Microtainer

5. Document results

   Record total volume of blood collected (for both microtainers if there was more than one fingerstick) for the hemoglobin test.

6. Give referral forms to women with a Hg of < 7.0 g/dL and children with a Hg < 8.0 g/dL.

7. Ensure safe-keeping of blood samples

   Store microtainer in upright position with lid tightly fastened

   Maintain microtainer cool (0 to 8 degree C) and dark until handover to Lab Tech before leaving the cluster.

NOTE:

1. ***Interviews may be performed by the nutritionist, lab technician, nurse or supervisor according to the needs of the team in the field.***
2. ***All team members are expected to assist one another as needed to complete all tasks in an efficient and timely manner. Only qualified professionals may draw blood or handle sample processing.***

Driver Job Description

Qualifications
Experienced driver, knowledgeable in basic vehicle repair.

Driver Responsibilities

1. Be familiar with the route required to reach identified clusters
2. Keep vehicle well-maintained and fueled at all times
3. Transport team members as required to ensure efficient collection of data
4. Ensure safe packing of the vehicle. Assure that vehicle is locked at all times in order to safeguard survey supplies and personal belongings of team members.

Note: All team members should assist one another as needed to complete all tasks in an efficient and timely manner. Only qualified professionals may draw blood or handle sample processing.

Appendix H

Field Teams

Field Teams

Copperbelt
Florence Mtwawale
Victoria Kalota
Christine Clewes (supervisor July)
Lizzie Sakada
Dilly Mwale (supervisor November)

Northern/Luapula
Kebby Mutale
Carol Ng'ona
Chitindi Sakala
Mutinta Ike
Catherine Mulikita (supervisor July)

Southern
Catherine Mukwangole
Mutinta Yumbe
Ward Siamusantu (supervisor July)
Mike Mwanza (supervisor November)
Linah Siazele

Eastern
Chipepo Kankasas (supervisor)
Juliet Ngambi
Rita Kakombo
John Chisoso

North western
Phillip Koni (supervisor)
Ulisa Tolopa Sikena
Kabwe Kabaso
Joan Mubanga

Western
Jean Welsh (supervisor July)
Astrida Chiya
Lungowe Nyaywa
Inonge Wamulume
Wilson Siasulwe (supervisor November)

Lusaka (administration and back-up team)
Eva Politzer
Chipo Mwela
Noah Mapundu

Appendix I

Questionnaire

Cluster | | | Province | | | Household # | | | |

QUESTIONNAIRE: NATIONAL VITAMIN A SURVEY – ZAMBIA

CDC/MOST/NFNC/UTH

SCREENING FORM FOR PARENTS/CAREGIVERS AND THEIR CHILDREN AGED 6 TO 59 MONTHS

I A. **Interviewer # [__-__]**	I B. **[_____/_____/_____]** **DD MM YY**

Introduction and Sample Selection –
To be read to Responsible Adult at Every Household

(Introduction of Interviewers. . .)

The Ministry of Health of Zambia is currently implementing a national survey to collect information about the health and nutrition of women and young children throughout the country. This information will be used to ensure that programs supported by the Ministry are helpful.

I C. **We would like to include your family in this study. May we tell you more about it and what will be needed?** Yes = 1

(If "No" STOP interview and proceed to next household) No = 2

(Record responses by filling in blanks where found [ ] or, where response options are listed after the question, by circling the number of the response given.)

I D. **To do the study we will need to ask about the health and nutrition of one of the children under the age of five and that of their mother or caregiver. Please tell us how many children between the ages of 6 months and 59 months (5 years) live in this household?**

Number of children [ ]

(*If "0" STOP interview and proceed to next household)*

Cluster | | | Province | | | Household # | | | |

I E. **What are the ages (in years or months) of each of these children? List their age in order of oldest to youngest.** *(Children less than 6 months or more than five years SHOULD NOT be listed. Include only those children born between August 1997 and January 2003)*

	Age
Child 1	_____ years ____ months
Child 2	_____ years ____ months
Child 3	_____ years ____ months
Child 4	_____ years ____ months
Child 5	_____ years ____ months
Child 6	_____ years ____ months
Child 7	_____ years ____ months
Child 8	_____ years ____ months
Child 9	_____ years ____ months
Child10	_____ years ____ months

I F. (*Use the random number table below to select the child that will participate in the survey. Once selected, circle the child's number in the table above)*

Last digit on child's pre-printed label	**Total number of children 6-59 months in this household**										
	1	2	3	4	5	6	7	8	9	10	
0	1	1	1	1	1	1	1	1	1	1	
1	1	2	2	2	2	2	2	2	2	2	
2	1	1	3	3	3	3	3	3	3	3	
3	1	2	1	4	4	4	4	4	4	4	
4	1	1	2	1	5	5	5	5	5	5	
5	1	2	3	2	1	6	6	6	6	6	
6	1	1	1	3	2	1	7	7	7	7	
7	1	2	2	4	3	2	1	8	8	8	
8	1	1	3	1	4	3	2	1	9	9	
9	1	2	1	2	5	4	3	2	1	10	

I G. **We have selected by chance the** *(insert age of selected child)* **child as the one to be included in this study. What is his/her first name?**

Name [_____]

I H. **Gender of child**: Male = 1
Female = 2

I I. **Are you the mother or (female) caregiver responsible for the care of this child on a regular basis?** *(If yes, go to Section II, Informed Consent)* Yes, Mother = 1
(If yes, go to Section II, Informed Consent) Yes, Caregiver= 2
No = 3

I J. **If possible, we would like to complete the survey by speaking to the mother or usual, female caregiver of (name of child). Is she available to speak with us now?**

(If yes, go to Section II, Informed Consent) Yes = 1
No, she is not available = 2
(If none, go to Q #11 for male caregiver) No mother/female caregiver exists = 3

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I K. **Could you tell us when she is expected to return today? We would like to make a return visit to speak with her.** *(If she is expected to be available today, make a total of 3 attempts to interview the mother or caregiver before selecting an alternate household)*

I K.a. Not available during first interview attempt
Time of expected return? [_____] = 2
(If not expected today, skip household and replace with the next) Not expected today = 3

I K.b. Interview attempt 2
(If yes, go to Section II, Informed Consent) Available = 1
Time of expected return? [_____] Not available = 2
Not expected to return today = 3

I K.c. Interview attempt 3:
(*If yes, go to Section II, Informed Consent)* Available = 1
(If "Not available" STOP interview and proceed to next household) Not available = 2

I L. **Are you the regular caregiver for this child?**
(If yes, go to Section II, Informed Consent) Yes = 1
No = 2

I M. **Is the regular caregiver available? We would like to speak with him now**.
(If yes, go to Section II, Informed Consent) Yes = 1
No = 2

I N. **Could you tell us when he is expected to return today? We would like to make a return visit to speak with him.** *(If he is expected to be available today, make a total of 3 attempts to interview him before selecting an alternate household)*

I N a. Not available during first interview attempt. Time of expected return? [_____] = 2
Not expected to return today = 3
(If he is not expected to return today, skip this household and proceed to the next)

I N b. Interview attempt 2 *(If available to Sect. II, Informed Consent)* Available Yes= 1
Time of expected return? [_____] Not available = 2
Not expected to return today = 3

I N c. Interview attempt 3: Available Yes = 1
(If "Not available" on 3rd visit STOP interview. Go to next household) Not available = 2

INFORMED CONSENT

We have a consent form that describes the study in detail. I would like to read this consent form to you. I will then ask if you are willing to participate in the study. (Proceed to read the consent form to the mother or caregiver. If consent is given, obtain a signature or thumbprint to confirm it. Consent must be documented before beginning the interview)

I P. Informed consent obtained. Yes = 1
(If "No" STOP interview and proceed to next household) No = 2

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NATIONAL VITAMIN A SURVEY – ZAMBIA
CDC/MOST /NFNC/UTH

DATA COLLECTION QUESTIONNAIRE FOR CHILDREN AGED 6 TO 59 MONTHS AND THEIR PARENT OR CAREGIVER

I A. **Interviewer # [__-__]** I B. **Date [_____/_____/_____]** **DD MM YY**	n=38 n= Place Child's Label Here

Proceed with interview only if informed consent given.

I. FAMILY DEMOGRAPHIC INFORMATION

We will begin the survey by asking some general information regarding your household.

1. **Is the head of this household male or female?**	Male = 1 Female = 2
2. **What is the main language that you speak at home?**	*(select only one option)* Bemba = 1 Nyanja=2 Lunda = 3 Luvale = 4 Kaonde = 5 Lozi = 6 Tonga = 7 Other, please specify_____ = 8 Don't know = 9

II. CHILD INFORMATION

Now we will ask you several questions related to the diet and health of (*name of randomly selected child)***. Please show us his/her Under Fives Health Card if you have one.**

3. **What year and month was** *(name of child)* **born?**	*Check if Under Five Card for birth date of child verified* [----]	Year [_ _ _ _] Month [_ _] Don't know = 9
4a**. In the last 2 weeks was** *(name of child)* **sick with fever . . . ?**		Yes = 1 No = 2 Don't know = 9
4b. **. . . cough/running nose?**		Yes = 1 No = 2 Don't know = 9
4c. **. . . diarrhea?**		Yes = 1 No = 2 Don't know = 9

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5. **Has this child been de-wormed in the past 6 months?**	*Check if Under Five Card for mebendazole dose was verified [ ]*	Yes = 1 No = 2 Don't know = 9
6. **How old was** *(name of child)* **when he/she was first fed something other than breastmilk (including water, formula, juice, semi-solid foods, etc.)?**		Age fed other fluids/foods [_ _ months] = 1 *(Go to Q# 8)* Never breastfed = 2 *(Go to Q# 8)* Still exclusively breastfed = 3 Don't know = 9
7. **How old was** *(name of child)* **when he/she stopped breastfeeding?**		Age stopped breastfeeding [_ _ months] = 1 Still being breastfed = 2 Don't know = 9
8. **Have you heard of Child Health Week, the week of special health events for children that happens twice each year?**		Yes = 1 (*If "No" go to Q #11)* No = 2 (*If "Don't know" go to Q #11)* Don't know = 9
9. **Did YOU take** *(name of child)* **for Child Health Week activities in June?**		Yes = 1 (*If "No" go to Q #11)* No = 2 (*If "Don't know" go to Q #11)* Don't know = 9
10. **Please describe what happened when you took your child to the Child Health Week?**		*(Circle all mentioned)* Red or blue vitamin A capsule mentioned =1 Remember cutting of capsule = 2 Correct distribution site mentioned = 3 Growth monitoring and promotion = 4 Immunization received = 5 Health education received = 6 Other child survival intervention received = 7 Don't know = 9
11. **Did** *(name of child)* **receive vitamin A during the special health week for children, Child Health Week, which took place in June?**		Yes = 1 No = 2 Don't know = 9
12. **Did this child receive vitamin A during an earlier Child Health Week, in January this year?**		Yes = 1 No = 2 Don't know = 9
13. **Since January, did** *(name of child)* **receive vitamin A at any time other than during Child Health Weeks between January and now?**	*Verify Vitamin A doses on Under Five Card [……]*	Yes = 1 No = 2 Don't know = 9
14. **How many doses did he/she receive from January up to today?**	*Verify Vitamin A doses on Under Five Card [.....]*	# doses [_ _] Don't know = 9

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15. **In what month or months, from January to today, did he/she receive a dose of vitamin A?**	*Verify Vitamin A doses on Under Five Card [….]* January [ ] August [ ] February [ ] September [ ] March [ ] October [ ] April [ ] May [ ] June [ ] July [ ] Don't know = 9
16. **Please estimate the total number of times in his/her life that** *(name of child)* **has been to a Child Health Week?**	1st visit 2ndvisit 2003 [ ] [ ] 2002 [ ] [ ] 2001 [ ] [ ] 2000 [ ] [ ] 1999 [ ] [ ] 1998 [ ] [ ] Don't know = 9 Never been to Child Health Week [ ]

III. **MOTHER/CAREGIVER INFORMATION (Go to Section IV if respondent is a male caregiver or a female caregiver under age 15 or older than 49 years)**
We will now ask a series of questions about your health and nutrition.

17. **What is the highest level of school you attended: primary, secondary, higher or none?**	Primary school = 1 Secondary school = 2 *(If "Higher", go to Q #19)* Higher =3 *(Go to Q #19)* None/no formal education = 4 *(Go to Q #19)* Don't know = 9
18. **What is the highest grade you completed at that level?**	Enter grade number here[_ _] Don't know = 9
19. **What is your age?**	Record the age in years and circle below [_ _] *(If 0 – 14, go to Section IV)* 0 to 14 years = 1 15 to 49 years = 2 *(If 50+, go to Section IV)* 50 years or more = 3
20. **Are you currently pregnant?**	*(If "Yes", continue with interview but remember that no blood sample will be drawn from pregnant women)* Yes = 1 No = 2 Don't know = 9
21. **What month and year did you deliver your LAST CHILD?**	Month [_ _] and Year [_ _] = 1 No children delivered= 2
22. **Are you currently practicing family planning by taking birth control pills, wearing Norplant, or receiving Depo injections?**	Yes = 1 No = 2 Other birth control = 3 Don't know = 9

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23a. **In the last 2 weeks have you been sick with fever . . . ?**	Yes = 1 No = 2 Don't know = 9
23b. **. . . cough/runny nose?**	Yes = 1 No = 2 Don't know = 9
23c. **. . . diarrhea?**	Yes = 1 No = 2 Don't know = 9
24. **When you were pregnant with your last child, did you have difficulty seeing during the day?**	Yes = 1 No = 2 Never pregnant = 3 Don't know = 9
25. **When you were pregnant with your last child, did you suffer from difficulty seeing at night or night blindness?**	Yes = 1 No = 2 Never pregnant = 3 Don't know = 9
26. **Did you receive a Vitamin A capsule like this** *(show mother the red Vitamin A capsules)* **in the two months following the birth of your last child?**	Yes = 1 No = 2 No births = 3 Don't know = 9

IV. SUGAR CONSUMPTION

Next we would like to ask some questions about some of the foods your family eats.

27. **Can you name any foods available in the market in Zambia that have Vitamin A added to them?**	*(Circle all mentioned)* Sugar = 1 Oil = 2 Other, please specify_____ = 3 *(If "No" go to Q #29)* No foods are fortified = 4 Don't know = 9
28. **Do you normally buy sugar that is fortified with Vitamin A?**	Yes = 1 No = 2 Don't know = 9
29**. Please estimate the number of days in the past month that you have had sugar in the house?**	Number of days with sugar [_ _] Don't know = 9
30. **Does your family take more sugar at some times during the month than at other times?**	Yes = 1 *(Go to Q #32)* No = 2 Don't know = 9
31. **When during the month does your family consume the most sugar, at the beginning, middle or end of the month?**	*(Circle all mentioned)* Beginning of the month = 1 Middle of the month = 2 End of the month = 3 Don't know = 9

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32. **Does your family consume less, more, or the same sugar during the rainy season compared to the dry season?**	Less = 1 More = 2 Same = 3 Don't know = 9

Now I would like to ask you about the home-prepared foods and drinks containing sugar that were taken yesterday.

(Read this following paragraph if the respondent is a mother or female caregiver between ages of 15 and 49)
I will begin by asking you to tell me which of the following sugar-sweetened, home-prepared, foods and drinks that you took yesterday from the time you woke in the morning until before going to sleep last night. I will then ask you to tell me the number of servings of each that you took yesterday. For tea, mealie meal and other cereals, I will ask you to estimate the amount of sugar you added for one serving. When we have completed your list I will ask the same questions regarding the foods and drinks taken by *(name of child)* **yesterday.** *(Go to Question #33).*

(Read this paragraph if the respondent is a male or a female aged less than 15 years or more than 49 years)
First, I would like you to tell me which of the following sugar-sweetened foods and drinks were taken by (*insert name of child)* **yesterday. Then, I will ask you the number of serving taken of each yesterday. For tea, mealie meal and other cereals, I will also ask you to estimate the amount of sugar you added for one of his/her servings.** *(Go to Question #34)*

Mother or Female Caregiver-- Sweetened Food Intake. Use either spoons or cups to estimate amount of sugar used by household: e.g. 2 x no. 2 spoon, 1x no.3 cup

33. **At anytime yesterday, did you take . .** *(Read each food and drink listed below and circle" yes" if they took it yesterday and "no" if they did not)*	**How many servings did you take yesterday** *("DK" for don't know)*	**Spoon size Use standard measures to estimate amount sugar used Write spoon number 1-6**	**Cup size Use standard cups provided To estimate sugar used Write cup number 1-5**
33a. **Tea with sugar?** Yes No			
33b. **M. meal/porridge w/ sugar** Yes No			
33c. **Other cereal with sugar?** Yes No			
33d. **Beans or bean soup w/sugar** Yes No			
33e. **Sour milk with sugar?** Yes No			
33f. **Scones?** Yes No			
33g. **Fritters?** Yes No			
33h. **Sweet Potatoes with sugar** Yes No			
33i. **Traditional brew with sugar** Yes No			
33j. **Anything else with sugar?** Yes No *(List food/drink)* _____			

Use this space to list additional foods and amounts of sugar (if needed):

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Child--- Sweetened Food and Drink Intake

34. **Yesterday, did** *(insert name of child)* **take . . .** *(Read each food and drink listed below and circle" yes" if the child took it yesterday and "no" if they did not)*	*(If "yes")* **How many servings did he/she take yesterday?** *("DK" for don't know)*	**Spoon size Use standard measures to estimate amount sugar given to child. Write spoon number 1-6**	**Cup size Use standard cups to estimate sugar given to child Write cup number 1-5**
34a. **Tea with sugar** Yes No			
34b. **Mealie meal with sugar** Yes No			
34c. **Other cereal with sugar** Yes No			
34d. **Beans or bean soup w/sugar** Yes No			
34e. **Sour milk with sugar** Yes No			
34f. **Scones** Yes No			
34g. **Fritters** Yes No			
34h. **Sweet Potatoes with sugar** Yes No			
34i. **Traditional brew with sugar** Yes No			
34j. **Anything else with sugar** Yes No *(List food/drink)* _____			

V. SUGAR AVAILABILITY IN THE HOUSEHOLD

As much of the sugar sold in Zambia is now fortified with vitamin A, we would like to know more about the sugar purchased for the family in the past 30 days?

35. **What brand or type of sugar did you purchase in the past 30 days?** *(circle "yes" for all mentioned and "no" if not mentioned)*	**Which size package did you purchase?** *(Record "DK" for don't know)*	**Number of packages purchased?**
35a. *Zambia Sugar* Yes No		
35b. *Kalungwishi* Yes No		
35c.Imported sugar *Yes No*		
35d. Unknown brand-repackaged *Yes No*		
35e. *Unknown brand- from open sack* Yes No		
35f. *Unknown brand –original package* Yes No		
35g.*Other (specify)* Yes No		
36. **Do you have any sugar available in the home today?**	Yes = 1 *(If no, go to Section VI)* No = 2 Don't know = 9	
37. **May I see ALL of the sugar that you have available?**	Yes = 1 *(If no, go to Section VI)* No = 2 Don't know = 9	

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38. **Observe all of the sugar available in the household to complete the table below. (circle" yes" for the brand or type of sugar available and "no" if not available)**		*Observe and record the amount of each sugar available in household (use standard spoons and cups to estimate amount)*	*Does the package have a label indicating that it is fortified with Vitamin A?*
38a. *Zambia Sugar*	Yes No		Yes No
38b. *Kalungwishi*	Yes No		Yes No
38c. Imported sugar	*Yes No*		Yes No
38d. Unknown brand-repackaged	*Yes No*		Yes No
38e. *Unknown brand- from open sack*	Yes No		Yes No
38f. *Unknown brand –original package*	Yes No		Yes No
38g.*Other (specify)*	Yes No		Yes No

VI. SAMPLE COLLECTION

(If sugar available in the household read. . .)

With your permission, we would like to take a small cup of sugar (>100g if available) that you use to prepare the food and drinks for the family. It will be tested to determine if it contains nutrients that are important for your health. We will replace the sugar with a 1kg packet of fortified sugar. Do you agree to provide the samples?

39. **May we take a small cup of sugar?**	Yes = 1 *(Go to Q#41)* No = 2 *(Go to Q#41)* None available = 3
40. (If sugar sample provided, tick the type or brand given)	*Zambia Sugar* [ ] Kalungwishi [ ] *Imported sugar* [ ] *Unknown brand-repackaged* [ ] *Unknown brand-loose from open sack* [ ] *Unknown brand –original package* [ ] *Other, Specify type* _____ [ ]

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We would now like to collect a small sample of blood. We will require only a small amount that we can take from the finger. This will help us to determine if families in Zambia are receiving sufficient amounts of Vitamin A which is important to maintaining good health.

41. **May we take the sample from your finger?**	Yes = 1 *(If "no", go to Q # 44)* No, refused = 2 *(Blood sample not requested- pregnant)* = 3
42. **May we take the sample from the finger of** *(name of child)***?**	Yes =1 *(If child not currently available, return twice more to the household to attempt to draw his/her blood)* No = 2

My colleague is a trained nurse. She will now come to take the blood sample. Thank you for your cooperation with the survey.

43. **Select the language in which the survey was conducted.**	Bemba = 1 Nyanja = 2 Lunda = 3 Luvale = 4 Kaonde = 5 Lozi = 6 Tonga = 7 Other, please specify_____ = 8 Don't know = 9

NOTE: BLOOD SAMPLES ARE NOT TAKEN FROM PREGNANT WOMEN OR MEN.
****

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Nurse's task completion checklist (initial if completed).

Activity	Completed	Results
Mother/caregiver		
Volume of blood collected in microtainer #1		_ _ _ µl
Volume of blood collected in microtainer #2 (if 2nd fingerstick was needed)		_ _ _ µl
Number of finger sticks done		
Malaria slide done		
Mother/caregiver's hemoglobin		_ _. _ g/dl
Referral given if anemic		
Child		
Volume of blood collected in microtainer #1 Volume of blood collected in microtainer #2 if 2nd fingerstick needed		_ _ _ µl _ _ _ µl
Number of finger sticks done		
Malaria slide done		
Child's hemoglobin		_ _. _ g/dl
Labels		
Pre-printed labels applied to questionnaire, malaria slide, microtainer and shipping manifest		

Interviewers' task completion checklist (initial if completed).

Activity	Completed	Results
Informed consent obtained		
Questionnaire fully completed		
Sugar sample collected		

Lab Technician task completion checklist (initial if completed and note date).

Activity	Completed	*Date*
Assure proper storage of samples in the field		
Process and assure proper storage of samples after processing		
Label samples correctly		
Pack samples for transport to Lusaka		

Assure receipt of samples at UTH

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Supervisor's task completion checklist (initial if completed and note date).

Activity	Completed	Date
Field review of questionnaire		
Office review of questionnaire		
First data entry		
Arrange for community health workers assistance and payment directly to the worker		

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