

Indicators for assessing Vitamin A Deficiency and their application in monitoring and evaluating intervention programmes



***INDICATORS FOR ASSESSING
VITAMIN A DEFICIENCY
AND THEIR APPLICATION IN
MONITORING AND EVALUATING
INTERVENTION PROGRAMMES***



World Health Organization

Reprinted in 1998 with the following adjustments:

Paragraphs renumbered from paragraph 29 onwards

Table 7: minimum sample size for 0.01 prevalence, from 153 650 to 153 640

Table 8: minimum sample size for $\geq 1\%$ - $<5\%$, from 4706 and 753 to 9508 and 1522

Annex 1: updated

© World Health Organization 1996

This document is not a formal publication of the World Health Organization (WHO), and all rights are reserved by the Organization. The document may, however, be freely reviewed, abstracted, quoted, reproduced or translated, in part or in whole, but not for sale or for use in conjunction with commercial purposes.

The views expressed in documents by named authors are solely the responsibility of those authors.

ACKNOWLEDGMENTS

WHO wishes to thank the Task Force "Sight and Life" for the financial support that made the production of this document possible; UNICEF for its financial contribution towards convening a technical consultation on the subject in 1992; and the many who took time to read and comment on a review version of this document whose suggestions are reflected herein. Grateful acknowledgment is also due to Eileen Brown for her tireless efforts in revising the manuscript, and to James Akre for his editorial assistance; both are from WHO's Programme of Nutrition, Geneva.

TABLE OF CONTENTS

Acknowledgments	iii
List of tables	vii
List of annexes	viii
Abbreviations	viii
INTRODUCTION	1
Background	1
PART I. SUMMARY OF INDICATORS FOR VAD SURVEILLANCE	5
1.1 Assessing VAD prevalence	5
1.2 Identifying high-risk areas/populations for interventions	8
1.3 Measuring progress towards long-term micronutrient goals	12
1.4 Monitoring and evaluating VAD control programmes	14
PART II. USING INDICATORS FOR VAD SURVEILLANCE	16
2.1 Purposes of VAD surveillance	16
2.2 Factors in selecting groups to measure	16
2.3 Appropriate groups to measure	17
2.4 Selecting indicators	19
2.5 Availability and interpretation of reference data	20
2.6 Presentation and interpretation of survey results	20
2.7 Definition of a public health problem	21
PART III. BIOLOGICAL INDICATORS OF VITAMIN A STATUS	22
3.1 Clinical indicators: Xerophthalmia	22
3.2 Subclinical indicators	24
3.2.1 Functional indicator: Night blindness	24
3.2.2 Biochemical indicators	26
Serum retinol	26
Breast milk vitamin A concentration	30
Relative dose response tests (RDR and MRDR)	34
Serum 30-day dose response test (+S30DR)	38
3.2.3 Histological indicator: Impression cytology	39
PART IV. ECOLOGICAL AND RELATED INDICATORS ASSOCIATED WITH RISK OF VAD	45
4.1 Purpose and use of indirect indicators associated with VAD	45
4.2 Nutritional status and diet-related indicators	46

4.2.1	Breast-feeding patterns	46
4.2.2	Anthropometric indicators of PEM	46
4.2.3	Prevalence of low birth weight (LBW)	47
4.2.4	Market and household food availability	47
4.2.5	Dietary patterns of vulnerable groups	48
4.2.6	Semi-quantitative and qualitative measures of food consumption	49
4.2.7	Beliefs and attitudes concerning food	50
4.3	Illness-related indicators	50
4.3.1	Immunization coverage rates	51
4.3.2	Measles case fatality rate	51
4.3.3	Disease prevalence rates	51
4.4	Socioeconomic indicators	53
4.4.1	Maternal education and literacy	53
4.4.2	Income/employment	53
4.4.3	Water supply and sanitation	54
4.4.4	Access to health and social services	54
4.4.5	Access to land	55
4.4.6	Access to agricultural services and inputs	55
PART V.	INTEGRATED APPROACHES TO CONTROLLING VAD	58
5.1	Improving dietary quality and quantity through dietary diversification	58
5.2	Improving dietary quality through food fortification	59
5.3	Supplementation	59
5.4	Public health measures	60

List of Tables

1.	Indicators of clinical vitamin A deficiency - xerophthalmia - in children 6-71 months of age	6
2.	Biological indicators of subclinical vitamin A deficiency in children 6-71 months of age	7
3.	Types of indicators useful for achieving broad surveillance objectives	9
4.	A. Relative ranking on a population base of some biological indicators useful for various surveillance purposes	10
	B. Ecologic indicators of areas/populations at risk of VAD	11
5.	Core indicators for assessing progress towards the goal of virtual elimination of VAD by the year 2000	13
6.	Examples of process indicators for monitoring and evaluating VAD control programmes	15
7.	Classification of xerophthalmia, minimum prevalence criteria and minimum sample sizes for defining clinical VAD of public health importance in a community of children under 6 years of age	23
8.	Prevalence of night blindness in children 24-71 months of age and minimum sample sizes for identifying a VAD public health problem	26
9.	Prevalence of serum values of vitamin A $\leq 0.70 \mu\text{mol/l}$ in children ≥ 1 year and minimum sample sizes for identifying a VAD public health problem	30
10.	Prevalence of breast-milk values $\leq 1.05 \mu\text{mol/l}$ ($\leq 8 \mu\text{g/g}$ milk fat) in a population of lactating women and minimum sample sizes for identifying a VAD public health problem	33
11.	Prevalence of a positive RDR or MRDR and minimum sample sizes for identifying a VAD public health problem	37
12.	Prevalence of positive +S30DR value in children 1-6 years of age and minimum sample sizes for identifying a VAD public health problem	39
13.	Comparison of the sensitivity and specificity of impression cytology against different indicators of VAD	42
14.	Prevalence of abnormal impression cytology in children 24-71 months of age and minimum sample size for identifying a VAD public health problem	44
15.	Ecological indicators and their potential uses in VAD surveillance	57

List of Annexes

Annex 1	Countries categorized by degree of public health importance of vitamin A deficiency, by WHO region	61
Annex 2	Sample form for weekly frequency of complementary feeding by food categories; Sample evaluation form guide	63
Annex 3	Sample of survey form for individual child/family	64
Annex 4	Minimum sample sizes for anticipated prevalence with relative precision of 20% and 50% at the 95% confidence level	65
Annex 5	List of participants	66

Abbreviations

CFR	case fatality rate	KAP	knowledge, attitudes and practice
CIC	conjunctival impression cytology	LBW	low birth weight
DA	dark adaptation	MCH	maternal and child health
DD	diarrhoeal disease	MRDR	modified relative dose response test
DGLV	dark green leafy vegetables	ORS	oral rehydration solution
DR	didehydroretinol	PEM	protein-energy malnutrition
EPI	expanded programme on immunization	PHC	Primary Health Care
HPLC	high pressure liquid chromatography	R	retinol
ICT	impression cytology with transfer	RDI	recommended dietary intake
IDA	iron deficiency anaemia	RDR	relative dose response test
IDD	iodine deficiency disorders	S30DR	serum 30-day dose response
IEC	information, education, communication	TSH	thyroid stimulating hormone
IMR	infant mortality rate	VAD	vitamin A deficiency
		VRT	vision restoration time
		WT/HT	weight for height

I. INTRODUCTION

Background

1. The global momentum for eliminating preventable debilitation, misery and death resulting from vitamin A deficiency (VAD) has significantly increased in the last decade. Heads of state, ministers, and other representatives of countries attending The World Summit for Children (New York, 1990), the Policy Conference on Ending Hidden Hunger (Montreal, 1991), and the International Conference on Nutrition (Rome, 1992) all pledged to virtually eliminate vitamin A deficiency and all its consequences, including blindness.¹ Global, regional and national plans are required to achieve this goal by the year 2000.

2. Vitamin A, in addition to the essential role it plays for vision and eye health, is now recognized as a critical factor in child health and survival. Traditionally, clinical signs and symptoms of xerophthalmia, sometimes supported by evidence of very deficient blood values and dietary intakes of the vitamin, have been used to identify populations with a deficiency of vitamin A. Furthermore, recent studies suggest that ill health and risk of death from some infections are also increased even in children who are not clinically deficient, but whose vitamin A body stores are depleted. Although there are several biological indicators for identifying subclinical (sometimes referred to as marginal) deficiency, none that is usable under usual field survey conditions is both adequately sensitive and specific to be used by itself.

3. There is thus an urgent need to provide guidelines for the selection, and appropriate use and interpretation, of practical indicators to identify areas where VAD is likely to occur, to assess vitamin A status in the areas/populations, and to monitor and evaluate programmes designed to eliminate the problem as a public health concern. The indicators selected should be *feasible* in terms of their cultural acceptability, their obtainability under field conditions, and their measurability at reasonable cost. In addition, the indicators should act with sufficient sensitivity and specificity to assess reliably the magnitude and severity of the problem.

4. The strengths and limitations of available biological indicators need to be examined to provide guidance for their selection and use under different conditions and for different purposes. Cut-off points and prevalence of deficient values to identify deficient populations are required to establish when a public health problem exists and at what level of concern, i.e. mild, moderate and severe.

¹ Participants in these meetings also agreed to eliminate iodine deficiency and to reduce substantially iron deficiency anaemia during the same time frame.

5. Frequently, resources are limited for using biological indicators to assess vitamin A status. In such situations, there is need for guidance in selecting, using and interpreting non-specific indicators to identify high-risk populations for targeting interventions.

6. When a decision to intervene is made, indicators are needed to measure efficacy and effectiveness of the intervention and to monitor progress towards eliminating VAD as an important public health problem.

7. In view of these needs, a technical consultation was convened (Geneva, 9-11 November 1992) with the following objectives and scope:

Objectives

- To identify appropriate indicators and establish cut-off points for assessing subclinical vitamin A deficiency in populations;
- To determine which indicator, or combinations of indicators, may be useful in identifying populations with vitamin A deficiency at levels that pose an important public health problem;
- To discuss, according to age and/or sex, which groups are most appropriate for assessment using different indicators;
- To consider the characteristics of the indicators and their usefulness given different surveillance objectives.

Scope

8. Indicators that define clinical vitamin A deficiency, i.e. signs of xerophthalmia reinforced, when available, by evidence of very deficient blood levels of vitamin A ($<0.35 \mu\text{mol/l}$) and their prevalence to determine a public health problem were not changed from those agreed upon in WHO Technical Report Series 672, 1982.² Although these indicators were not reviewed in detail as a part of this consultation, they are included in this report for the sake of completeness. In view of recent evidence associating morbidity and mortality with VAD, and the well-recognized "clustering" of VAD, any xerophthalmia in an area warrants careful consideration as an important public health problem. Decreased morbidity and mortality can be expected by using any combination of interventions to improve vitamin A status³ in such populations⁴.

² *Control of vitamin A deficiency and xerophthalmia*. Report of a joint WHO/UNICEF/USAID/Helen Keller International/IVACG meeting, 1982, Technical Report Series 672, World Health Organization, Geneva, 1982.

³ Subcommittee on Nutrition. *Controlling vitamin A deficiency*. A report based on the ACC/SCN Consultative Group Meeting on Strategies for the Control of Vitamin A Deficiency, 28-30 July 1993, Ottawa, Canada. ACC/SCN State-of-the Art Series. Nutrition Policy Discussion Paper No. 14, January 1994.

⁴ Beaton et al. *Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries*. ACC/SCN State-of-the-Art Series, Nutrition Policy Discussion Paper No. 13, December 1993.

9. Subclinical VAD, which was the subject of the consultation, is defined as:

tissue concentrations of vitamin A low enough to have adverse health consequences, even if there is no evidence of clinical xerophthalmia.

In the light of recent findings, the consultation agreed that the term *deficiency* includes clinical as well as subclinical VAD (severe, moderate and mild degrees), all levels of which are likely to adversely affect health. In situations where deficiency refers only to the presence of clinical signs, the consultation recommended that the term clinical deficiency or xerophthalmia should be used.

10. The specific biological indicators of VAD considered were:

Functional indicator: night blindness.

Biochemical indicators: serum retinol; breast-milk vitamin A; serum retinol binding protein; and the various dose response measurements: relative dose response (RDR), modified relative dose response (MRDR), and the serum 30-day response (+ S30DR).

Histological indicators: conjunctival impression cytology (CIC); impression cytology with transfer (ICT).

11. A composite of non-specific but supportive ecologic and demographic indicators was also suggested that can be useful in mapping or locating where VAD areas/populations are likely to be found. These indicators included:

Nutritional status and diet: breast-feeding patterns; anthropometric indicators of PEM; prevalence of low birth weight; market and household food availability; dietary patterns of vulnerable groups; semi-quantitative and qualitative indicators of food consumption levels; beliefs and attitudes concerning foods.

Illness and disease patterns: immunization coverage rates; measles CFR; disease prevalence rates.

Socioeconomic variables: maternal education and literacy; income and employment; water supply and sanitation; access to health and social services; access to land; and agricultural services and inputs.

Global distribution of VAD

12. WHO recently updated its information on the global prevalence of VAD and established a databank as part of the Micronutrient Deficiency Information System (MDIS), which also covers iodine and iron deficiencies. Based on the definition of VAD agreed upon in this consultation, and using the MDIS in consultation with WHO regional offices, countries with VAD are listed in Annex I based on the best current estimate of the level of the problem's public health importance. The categories severe, moderate or mild subclinical deficiency are based, respectively, on biochemical

evidence and reports of sporadic occurrences of xerophthalmia, and/or dietary and other supportive ecological evidence of high risk. Countries for which data are lacking are also listed, as are countries where VAD is under control or there is not likely to be a problem.

13. WHO recognizes the dynamic situation globally as countries newly assess their problem or update information available from monitoring VAD control programmes. To respond to these changes, the global situation will be updated periodically based on data made available to the MDIS. WHO appreciates receiving new data from countries, through its regional offices or directly to headquarters in Geneva, for incorporating into the MDIS database.

14. All global and country-specific information on which the prevalence of clinical and subclinical VAD is based is found in the MDIS Working paper #2⁵ published in 1995 and available from the WHO Nutrition Unit. WHO estimates that around 1995 nearly 3 million children 0-5 years of age are annually affected by xerophthalmia, and that an additional 251 million are severely or moderately subclinically deficient.

⁵ *The Global Prevalence of Vitamin A Deficiency*. MDIS Working paper #2, WHO/NUT/95.3, World Health Organization, Geneva, 1995.

PART I. SUMMARY OF INDICATORS FOR VAD SURVEILLANCE

15. Surveillance is the careful and continual oversight of a situation or population⁶ and may be undertaken for a number of reasons. Surveillance of VAD may be done to assess the magnitude, severity and distribution of the problem; to identify and characterize high-risk areas/populations where control programmes are needed; to track progress towards attainment of long-range goals; and/or to monitor progress and evaluate impact of control programmes. Indicators are derived from information that characterizes a situation or population; their careful selection and evaluation are thus critical to accurate surveillance. The purpose of VAD surveillance will determine indicators which are appropriate and the limits to interpreting data. Because the vitamin A status of populations can worsen as a result of prolonged adverse climatic conditions and significant economic and/or political changes, it is important that these factors be included as a part of surveillance systems. Indicator selection should be governed not only by technical and financial feasibility, but also be consistent with a given demographic and cultural context.

16. This section summarizes key purposes for VAD surveillance and indicators for this purpose recommended by the consultation, their cut-off points to define deficiency, and suggested prevalences of deficiency indicating a given level of public health concern. Later sections provide additional detailed information for consideration in selecting an indicator, including biological features, acceptability, technical feasibility and relative costs. For convenience, the biological indicators (clinical and subclinical), ecological indicators, core indicators for measuring progress toward elimination of VAD, and examples of process indicators for evaluating intervention programmes are provided in Tables 1-5.

1.1 Assessing VAD prevalence

17. A fundamental aim of VAD surveillance is to determine VAD's magnitude, severity and distribution. This is usually accomplished through surveys, which help to identify high-risk populations for targeting interventions while providing a baseline for monitoring change in vitamin A status over time. The data are also useful for highlighting the significance of VAD problems and stimulating action. Both clinical and subclinical biological indicators of vitamin A nutritional status are required for this purpose.

⁶ (a) *Methodology of Nutritional Surveillance*, Technical Report Series 593. World Health Organization, Geneva, 1976;

(b) Mason J, et al. *Nutritional Surveillance*, World Health Organization, Geneva, 1984.

18. Biological indicators of vitamin A status, cut-off levels that define deficiency, and minimum prevalence levels that define a public health problem are presented in Tables 1 and 2. In Table 1 only criteria for corneal disease (corneal xerosis, ulceration, keratomalacia and scars) directly document blinding, or immediately blinding, ocular disease.

Table 1

Indicators of clinical vitamin A deficiency-xerophthalmia-
in children 6-71 months of age
(Prevalence of any one or more indicators signifies a public health problem)^{a,b}

Indicator	Minimum Prevalence
Conjunctival xerosis/with Bitot's spot (X1B)	> 0.5%
Corneal xerosis/ulceration/keratomalacia (X2, X3A, X3B)	> 0.01%
Corneal scars ^c (XS)	> 0.05%

^a Night blindness (XN) is a symptom included in the classification of xerophthalmia together with the other clinical eye signs. The consultation reaffirmed that a prevalence of night blindness > 1.0% in children 24-71 months of age indicates a public health problem. In addition, a serum level of vitamin A (retinol) is often used with the clinical classification to provide supportive evidence of an important problem. A prevalence of >5% of serum levels <0.35 $\mu\text{mol/l}$ is strong corroborative evidence of any clinical criteria met to identify an urgent public health problem.

^b The consultation did not review prevalence rates for xerophthalmia that indicate a public health problem. Clinical signs and symptoms undoubtedly provide a gross underestimation of the beneficial effect that a vitamin A intervention might have on a population. Indeed, in view of recent findings from mortality and morbidity trials, any xerophthalmia in a population is worthy of careful review given its potential public health importance.

^c Lack of a history of traumatic eye injury or use of topical traditional medicines increase the specificity of this VAD indicator.

Table 2

Biological indicators of subclinical vitamin A deficiency
in children 6-71 months of age

Prevalence below cut-offs to define a public health problem and its level of importance			
Indicator (cut-off)	Mild	Moderate	Severe
<u>FUNCTIONAL</u>			
Night blindness I (present at 24-71 mo)	>0 - <1%	≥1% - <5%	≥5%
<u>BIOCHEMICAL</u>			
Serum retinol (≤0.70 μmol/l)	≥2 - <10%	≥10% - <20%	≥20%
Breast milk retinol (≤1.05 μmol/l or ≤8 μg/g milk fat.)	<10%	≥10 - <25%	≥25%
RDR (≥20%)	<20%	≥20 - <30%	≥30%
MRDR (ratio ≥0.06)	<20%	≥20 - <30%	≥30%
+ S30DR (≥20%)	<20%	≥20 - <30%	≥30%
<u>HISTOLOGICAL</u>			
CIC/ICT (abnormal at 24-71 mo of age)	<20%	≥20 - <40%	≥40%

There is a public health problem (the level of public health importance is indicated by the prevalences noted in the table) when:

the prevalence in a population of at least two of the above biological indicators of vitamin A status is below the cut-off;

or when

one biological indicator of deficiency is supported by at least four (two of which are nutrition and diet-related as in Table 4) of a composite of demographic and ecological risk factors such as:

- IMR > 75/1000 live births; under-5 year MR > 100/1000 live births;
- full immunization coverage or, particularly measles immunization coverage, in <50% of children at 12-23 months or age;
- <50% prevalence of breast-feeding in 6-month-old infants;
- median dietary intake <50% of recommended safe level of intake among 75% of children 1-6 years of age;
- two-week period prevalence of diarrhoea ≥20%;
- measles CFR rate ≥ 1%;
- no formal schooling for ≥50% of women 15-44 years of age;
- <50% of households with a safe water source.

19. The cut-off values suggested for demographic and ecologic risk factors are arbitrary. When information is available, these and similar factors reflecting social and economic deprivation and dietary inadequacy can be used for ranking areas/populations according to risk level in support of any biological criterion that identifies an important public health problem.

20. When a public health problem of mild, moderate or severe importance is identified, intervention is called for because health consequences are likely even at mild and moderate levels of public health importance. A contextual analysis is warranted to determine likely causes of the situation, and timely attention is required for allocating resources and taking action. However, the mix of interventions chosen may be influenced by the level of public health importance identified in order to balance short-term measures with sustainable long-term solutions.

1.2 Identifying high-risk areas/populations for interventions

21. Assessing factors associated with VAD is also critical to developing intervention programmes. This type of surveillance activity is concerned with selecting priority areas for intervention, thereby allowing for more efficient allocation of resources. Indicators that are useful for identifying high-risk areas also provide data reflective of the context for selecting, designing, implementing and evaluating effectiveness of the appropriate mix of control programmes. Table 3 evaluates the usefulness of various indicators for achieving broad types of surveillance objectives.

Table 3

Types of indicators useful for achieving broad surveillance objectives

Objective	Useful indicators
VAD status	Biological
Ranking area/population risk	Nutritional, illness, socioeconomic
Select priority areas/create mix of intervention strategies	Demographic, ecologic

22. Biological indicators are the most specific and useful for determining risk assessment, targeting programmes and evaluating their effectiveness. They are essential for evaluating the vitamin A status of a population. Table 4A suggests a relative ranking of the usefulness of biological indicators of subclinical VAD.

23. Where it is not feasible to obtain biological indicators, demographic and ecologic indicators may be sufficient to achieve some of the objectives if there is evidence that VAD is a problem of public health importance. Table 4B lists some of the most relevant of these indirect risk indicators. Before intervening, however, it is preferable to use a biological indicator to confirm the existence of VAD.

Table 4

A.. Relative ranking on a population base of some biological indicators useful for various surveillance purposes

Indicator	Risk assessment	Targeting programmes	Evaluating effectiveness
Night blindness	+++	+++	+++
Breast milk retinol (lactating mothers and breast-fed infants)	++	+++	++
Serum retinol	++	+	++
RDR/MRDR	+++	+++	+++
CIC/ACT	+	---	---
+S30DR	---	---	+++

B. Ecologic indicators of areas/populations at risk of VAD

Nutrition and diet-related indicators^a

Indicator	Suggested prevalence
Breast-feeding pattern <6 months of age ≥6 - 18 months of age	<50% receiving breast milk <75% receiving vitamin A-containing foods in addition to breast milk, 3 times/week
Nutritional status (< -2SD from WHO/NCHS reference for children < 5 years of age) Stunting Wasting	≥30% ≥10%
Low birth weight (<2500 g)	≥15%
Food availability Market Household	DGLV unavailable ≥6 months/yr <75% households consume vitamin A-rich foods 3 times/week
Dietary patterns 6 -71 months old children Pregnant/lactating women	<75% consume vitamin A-rich foods at least 3 times/week
Semi-quantitative/qualitative Food frequency	Foods of high vitamin A content eaten <3 times/week by ≥75% vulnerable groups

^a The suggested prevalence cut-off levels are arbitrary. However, greater weight should be given to this group of indicators in identifying high-risk populations than is given to the other ecologic indicators noted below.

Illness-related indicators in children 6-71 months of age^a

Indicator	Suggested prevalence
Immunization coverage at 12-23 months of age	<50% fully immunized or <50% immunized for measles
Measles CFR	≥1%
Reported diarrhoea disease rate (2 week period prevalence)	≥20%
Reported fever rates (2 week period prevalence)	≥20%
Helminthic infection rates, particularly ascariis	≥50%

^a The suggested prevalences are arbitrary, and are suggested only to assist in the relative ranking of population vulnerability. They are best used in association with a biological indicator and more than one nutrition and diet-related indicators.

Socioeconomic indicators:

Levels of maternal education and literacy
Income/employment
Water supply and level of sanitation
Access to health and social services
Access to land
Access to agricultural services/inputs

24. Socioeconomic indicators such as those noted above can provide supportive information. These indicators should be used with nutrition and diet and illness-related indicators to identify and rank areas or populations at risk of an important problem of VAD.

25. By themselves, none of these ecologic or related indicators are sufficient to determine if VAD actually exists in an area/population. Biological indicators must be used to verify vitamin A status.

1.3 Measuring progress towards long-term micronutrient goals

26. Many countries have pledged to work towards achieving a set of child health, nutrition and development goals by the year 2000. The relevant goal for vitamin A among the World Summit for Children Goals,⁷ which were also endorsed by the International Conference on Nutrition (ICN),⁸ is "the virtual elimination of VAD and all its consequences, including blindness." Surveillance activities can provide a quantitative basis for assessing progress towards meeting this goal.

27. The clinical indicators in Table 1 are useful for determining when the virtual elimination of blinding VAD has been achieved. The threshold values for biological indicators in Table 2 are appropriate where clinical VAD has been reduced but (applied to serum retinol) rates of infection remain high. The consultation selected the indicators in Table 5 as core indicators for determining progress in countries towards achieving the virtual elimination of all consequences of VAD. For this specific surveillance purpose, goals for threshold prevalence levels in serum differ from those in Table 2 (i.e. <5% rather than 10%). The lower prevalence was selected given that improving health and social conditions over time will reduce adverse factors (e.g. underlying acute and chronic infections) that lower serum values, and adverse socioeconomic factors that cause inadequate diets. Prevalence levels marking the achievement of long-term vitamin A goals therefore should reflect the prevalence expected in populations that enjoy not only good vitamin A status but also minimal levels of acute and chronic infections. Less than 5% with serum values $\leq 0.70 \mu\text{mol/l}$ is characteristic of affluent societies

⁷ First call for children. *World Declaration and plan of action from the World Summit for Children*, UNICEF, NY, 1990.

⁸ *World Declaration and Plan of Action for Nutrition*. FAO/WHO, International Conference on Nutrition, Rome, December 1992.

and children with adequate vitamin A status.⁹ Moreover, when diets are adequate in vitamin A, few mothers have breast milk values under 1.05 $\mu\text{mol/l}$.¹⁰

Table 5

Core indicators for assessing progress towards
the goal of virtual elimination of VAD by the year 2000

Functional indicator ^a	Prevalence goal
Night blindness (children 24-71 months of age)	<1%
Biochemical indicators:	
Serum retinol $\leq 0.70 \mu\text{mol/l}$ (children 6-71 months of age) or	<5%
Breast milk retinol $\leq 1.05 \mu\text{mol/l}$ or $\leq 8 \mu\text{g/g}$ milk fat	<10%

^a Other clinical indicators of xerophthalmia, i.e. conjunctival xerosis with Bitot's spots (X1B) <0.5%; corneal xerosis/ulceration/keratomalacia (X2,X3A,X3B) <0.01%; corneal scars (XS) <0.05%, where known to occur can also be used to assess progress towards eliminating VAD, and especially towards eliminating vitamin A-related blindness.

-
- ⁹ (a) Pilch S. M. Analysis of vitamin A data from the health and nutrition examination surveys. *Journal of Nutrition*, 1987, 117:636-40;
 (b) Flores H. et al. Serum vitamin A distribution curve for children aged 2-6 y known to have adequate vitamin A status: a reference population. *American Journal Clinical Nutrition*, 1991, 54:707-11.

¹⁰ Newman V. *Vitamin A and breastfeeding: a comparison of data from developed and developing countries*. Wellstart International, San Diego, CA, 1992.

I.4 Monitoring and evaluating VAD control programmes

28. Surveillance also extends to evaluation of effectiveness of programme implementation and impact. Indicators of programme effectiveness, i.e. process indicators, measure the functioning of programmatic activity necessary to have an impact on vitamin A status. Process indicators should be selected to measure activities critical to the specific control programme(s). Table 6 provides some examples of process indicators that might be used in some types of VAD control programmes. Programme coverage is critical to both this evaluation function and programme success. Evaluation of the impact of control programmes requires the use of biological indicators. Other authoritative documents provide more detail and examples of process indicators and their uses in monitoring and evaluation.¹¹

¹¹ (a) Arroyave G. et al. *Methodologies for monitoring and evaluating vitamin A deficiency intervention programs*. A report of the International Vitamin A Consultative Group (IVACG). The Nutrition Foundation, Washington, D.C., 1989.

(b) Program Against Micronutrient Malnutrition (PAMM) and the Micronutrient Initiative (MI) are developing a detailed manual for monitoring VAD intervention programmes which is expected to be available in 1996.

Table 6

Examples of process indicators for monitoring
and evaluating VAD control programmes

Supplement distribution programme
Percent coverage of at-risk population by programme, e.g. capsules (supplement) received and delivered/year/target population
Percent of the health centres/institutions etc. with sufficient supplement for coverage of the target population(s) for a 4-6 month distribution cycle
Fortification programme
Number of vitamin A-fortified foods available in selected markets
Percentage of fortified foods available in market that meet specified levels of vitamin A
Percentage of households containing a specific vitamin A-fortified food selected as suitable for consumption by target groups
Percentage of target group consuming a specific fortified food
Nutrition education/social marketing programmes
Number of contacts for message-delivery per week
KAP indicators of messages delivered and understood at the household level
Percentage awareness of consequences of VAD and local vitamin A-containing foods:
Percentage of under-24-month-old infants consuming the RDI of vitamin A from foods or supplements
Feeding patterns for vitamin A-rich foods, e.g. percentage of children under 3 years of age eating vitamin A-rich foods 3 times a week
Horticulture programme
Percentage of homes in a district with a garden having provitamin A-containing foods
Percentage of schools in a district with gardens having provitamin A-containing foods.

PART II. USING INDICATORS FOR VAD SURVEILLANCE

2.1 Purposes of VAD surveillance

29. Surveillance is the continual collection of information from which indicators have been derived that characterize the present situation and provide a baseline for determining future changes. Therefore, key to VAD surveillance are indicators which measure vitamin A status, the conditions that contribute to VAD, and how vitamin A status shifts over time in response to changing conditions. The appropriate composite indicators depend in part on the purpose(s) of VAD surveillance. Four of the most frequent purposes are: (a) assessment of the magnitude, severity and distribution of VAD prevalence; (b) identification of high-risk areas/populations; (c) tracking progress towards attainment of long-range goals; and (d) monitoring progress and evaluating impact of intervention and control programmes. The feasibility of obtaining reliable data for specific purposes should be considered not only within the constraints of available technical and financial resources, but also in the light of the specific demographic and cultural context. These considerations will determine the selection of appropriate indicators.

2.2 Factors in selecting groups to measure

30. A variety of age groups of both sexes could serve as a focus for VAD surveillance. The selection of the optimal group will depend upon the purpose for surveillance and other considerations, including vulnerability, representativeness and accessibility. When surveillance includes multiple nutrition and health problems in addition to VAD, the age group selected to achieve all the desired outcomes may not be optimal for surveillance of VAD alone.

31. Vulnerability. To serve as a sensitive indicator, the population selected must be vulnerable to the deficiency. Three aspects of vulnerability are:

- ◆ extent of exposure and responsiveness to deficiency;
- ◆ severity of health consequences of deficiency;
- ◆ degree of responsiveness to interventions.

32. Representativeness. Two aspects of representativeness are:

- ◆ Internal validity. Is the selected group representative of all persons in the same age/sex group in the community? For example, if preschool children examined in an under-five programme are selected, are they representative of all preschool-aged children in the community?

- ◆ External validity. Is the VAD status of the selected group representative of the status of other vulnerable groups in the community? It may be that the apparent prevalence and/or severity of VAD in the selected group (e.g. preschool-aged children) contributes to overestimating or underestimating the prevalence and/or severity in other vulnerable groups (e.g. lactating women).

33. Accessibility. Populations easily accessible for assessment, e.g. infants contacted through an immunization programme, children in growth-monitoring programmes, or women who attend maternal-child health (MCH) clinics, may facilitate surveillance and reduce logistical costs. However, the most accessible groups may not include a proportion of the most vulnerable group equal to that in the general population. Therefore, utmost caution should always be taken in extrapolating to the general population information obtained from easily accessible groups.

34. Usefulness for surveillance of multiple micronutrient and other health problems. Because nutrient deficiencies seldom occur in isolation, it may be economical if the group selected for VAD surveillance also serves for obtaining information relative to other nutritional and related health problems. For example, surveillance of VAD among preschool-aged children that use a consumption indicator of key vitamin A-rich foods in the diet might also serve to determine the consumption frequency of iron-containing foods (they may in fact be similar), and/or foods rich in ascorbic acid that are important for enhancing the bioavailability of iron or, concurrently, to determine the frequency, amount and kind of (iodized?) salt consumed.

2.3 Appropriate groups to measure

35. Newborns and infants under 6 months of age. In settings where breast-feeding predominates for at least 4-6 months, newborns and infants under 6 months of age are *not* a useful group for VAD screening. Although vitamin A stores are minimal at birth and blood levels tend to be low, stores climb rapidly in the breast-fed neonate whose mother is well-nourished and therefore has adequate vitamin A in her milk. Generally speaking, stores are maintained at an adequate level for at least 4-6 months while breast-feeding continues.¹² However, in settings where breast-feeding prevalence is low or of short duration, infants weaned early to diluted or non-fortified milks are highly vulnerable to severe clinical vitamin A deficiency as are non-breast-fed newborns given similar milks. Surveillance is best done by including a breast-feeding/dietary history to identify deprived populations where breast-feeding does not predominate for at least the first 6 months of life.

36. Infants >6 months and children up to 6 years. Although this age group is highly vulnerable to VAD and is the most useful for surveillance, accessibility may be a problem. Reliance on access through established community programmes may pose problems of representativeness. Measles

¹² (a) Wallingford JC, Underwood BA. Vitamin A deficiency in pregnancy, lactation, and the nursing child. In: Bauernfeind JC (ed) *Vitamin A Deficiency and Its Control*. Academic Press, Inc., NY, (1986);

(b) Newman V. *Vitamin A and breastfeeding: a comparison of data from developed and developing countries*. Wellstart International, 1993, pp 21-24;

(c) Underwood B. Maternal vitamin A status and its importance in infancy and early childhood. *American Journal of Clinical Nutrition*, 1994, 59:517S-24S.

vaccination may offer the highest accessibility and representativeness for the first contact in areas with high coverage for this vaccine. However, additional contact points for surveillance that cover the full age period of maximum vulnerability, i.e. up to 6 years of age, should be identified. This age group is also useful for surveillance of other nutrition problems such as iron deficiency anaemia, iodine deficiency disorders, and detection of malnutrition through anthropometry.

37. School-aged children. From six years of age onwards, children are less vulnerable to severe health consequences from VAD and some indicators (e.g. Bitot's spots) are less responsive to intervention programmes. Children in school are accessible and can serve as scouts for deficiency in younger siblings, as advocates for vitamin A-containing diets in their homes, for promoting long-term solutions through home and school gardening, and for receiving vitamin A-relevant nutrition education.

38. Pregnant women at MCH clinics. Pregnancy increases women's vulnerability only slightly as the fetus' additional daily needs are small. Requirements can be met through diet or, if necessary, supplements that do not exceed 10 000 IU daily throughout pregnancy. Surveillance is best done by taking a history for evidence of night blindness (where a local term for the condition exists) and/or applying one of the dose-response tests (MRDR or RDR). Serum retinol levels alone may be misleading due to the haemo-dilution that accompanies later stages of pregnancy. On the other hand, pregnant women are good candidates for surveillance of other nutrition-related health problems such as iodine deficiency disorders, iron deficiency anaemia and risk of delivering a low-birth-weight infant. Selection of the appropriate combination of VAD status indicators is particularly important to realize the potential cost-savings from surveillance contacts for multiple nutrient/health-related problems.

39. Lactating women preferably within 4-6 weeks (or at most 8 weeks) from birth. Vitamin A needs increase during lactation to replace daily losses in breast milk. Women in endemic vitamin A-deficient areas are priority candidates for receiving a high-dose supplement (200 000 IU) during the infertile period following delivery, thereby raising and maintaining the concentration of vitamin A in breast milk. Lactating women within the first, or at most the second, postpartum month may be accessible through the BCG/OPV vaccination contact where coverage rates are high, and/or when the infant is delivered by a health worker (potentially including trained traditional birth attendants).

40. Lactating women from 2 months onward and other fertile women. Malnourished lactating women are vulnerable to mild-to-moderate VAD because of the daily need to replace vitamin A secreted in breast milk. However, they should not receive vitamin A supplements in excess of 10 000 IU daily. Because the concentration of vitamin A in breast milk is a reflection of a woman's diet, it is a useful indicator for surveillance of VAD risk in populations where breast-feeding continues for a significant time as the infant's primary food source of vitamin A. Representativeness of the distribution of milk vitamin A levels is high if samples are obtained from randomly chosen mothers. Average breast milk vitamin A concentration, therefore, can be useful for identifying not only women and their breast-feeding infants at risk of VAD, but also for locating high-risk areas generally. Breast milk is often more easily obtained than blood and may be useful as well for surveillance of risk of other micronutrients deficiencies, e.g. iodine.

2.4 Selecting indicators

41. Indicators are basically of two types: outcome indicators, which provide a measure of impact on VAD status, and process indicators, which measure whether or not the intervention is functioning effectively. Outcome indicators may refer to exposure (for example breast-milk vitamin A as a reflection of the vitamin-A intake of a mother and her breast-feeding infant), or to impact, whether on morphology (as estimated by impression cytology), or function (as estimated by night blindness). Alternatively, outcome indicators can be categorized according to whether assessments are direct, i.e. functional or biochemical, or indirect, i.e. dietary or ecological. Once the population(s) selected for assessment are defined, the choice of particular indicators should be based on consideration of both the criteria discussed below and specific surveillance objectives.

42. Acceptability. The acceptability of a particular indicator may vary among different populations. Some measures are more widely accepted (e.g. dietary intake or breast milk vitamin A) than others (e.g. drawing venous blood). Acceptability is also an issue for field staff performing the tests. For example, drawing blood samples in populations with a high prevalence of HIV infection involves some level of risk, or perceived risk, that should be considered in selecting indicators.

43. Technical feasibility. Technical feasibility involves a number of factors including:

- ◆ ease of data or sample collection;
- ◆ sample storage and transport requirements;
- ◆ transportability and durability of field equipment;
- ◆ availability of specialized personnel to obtain and analyse specimens;
- ◆ availability of equipment, spare parts and maintenance personnel.

44. Cost. Costs associated with the use of certain indicators include:

- ◆ capital costs for facilities and equipment;
- ◆ recurring costs for supplies and reagents;
- ◆ maintenance costs;
- ◆ training costs;
- ◆ personnel, administrative and related expenses.

Overall, the cost per test tends to decrease as the number of tests increases.

45. Performance. Useful measures of indicator performance in identifying VAD include sensitivity, specificity and reliability. These performance attributes are best evaluated when there is an agreed reference standard against which performance can be compared. Clinical eye signs are a suitable reference standard for severe VAD but are rare occurrences that are insensitive to mild and moderate deficiency. Total body store is the relevant standard for biological indicators of vitamin A, but it is impractical to obtain on a population basis using current methodology. Thus, at present, there is no easily obtained indicator that is useful as a reference standard for VAD. The nearest practical approximation is an estimation of vitamin A liver stores measured indirectly using one of the dose-response tests, RDR or MRDR. Indeed, the dose response tests are increasingly being used as

a reference for other indicators of vitamin A status, even if these tests also have limitations which are discussed below.

2.5 Availability and interpretation of reference data

46. Interpretation of VAD status depends on the availability of reference data, preferably derived from populations known to have adequate vitamin A status, as from "elite" groups within the population itself. The presence of agreed reference data for a given indicator should guide the selection of indicators and groups for surveillance. (Where such data are unavailable, local control populations may be needed.) Standardized reference data enhance interpretation across different studies and assist in establishing cut-off values and prevalence levels for use in identifying public health problems and their severity. Cut-off levels for interpreting serum retinol distribution curves used until recently were not derived from population-based surveys with these characteristics, particularly for children. The National Health and Nutrition Examination Survey (NHANES) in the USA and in a limited population-based study before and after assuring adequate vitamin A status in Northeast Brazil¹³ comes nearest to reference population.¹⁴ Reference data are still being compiled for some of the newly identified subclinical biological indicators (breast milk retinol, dose response tests, CIC/ICT). This means that the criteria selected by the consultation based on current, limited experiences will need to be revised in the future as field experience using them increases.

2.6 Presentation and interpretation of survey results

47. Some indicators of VAD measured on a continuous scale (e.g. serum and breast milk vitamin-A levels) may not be normally distributed, particularly in deficient populations. Under these conditions the use of means and standard deviations is likely to be inappropriate. If possible, the full distribution of results should be presented, in addition to a measure of the central tendency (mean or median) and the use of cut-off points to describe the upper or lower range of the distribution. The percentage of individuals at the extremes of a distribution can be characterized by using standard cut-off points and tabulating the prevalence values above or below cut-off values. Several threshold values may be used to express the magnitude of the problem occurring at different points of the distribution and that reflect different levels of risk. For example, serum distributions lower cut-off points (e.g. $<0.35 \mu\text{mol/l}$) may be selected to highlight the most extreme cases, while higher cut-off points (e.g. $<0.70 \mu\text{mol/l}$ or $<1.05 \mu\text{mol/l}$) may be useful in identifying that proportion of the population possibly at risk of inadequate vitamin A status even if it is not necessarily severely deficient.

¹³ Flores H, et al. Serum vitamin A distribution curve for children aged 2-6 y known to have adequate vitamin A status: a reference population. *American Journal of Clinical Nutrition*, 54:707-11.

¹⁴ Pilch, SM. Analysis of vitamin A data from the health and nutrition examination surveys. *Journal of Nutrition*, 1987, 117:636-40.

2.7 Definition of a public health problem

48. A public health problem exists when the prevalence of observations below a cut-off point that defines deficiency is considered unacceptable. Prevalences to identify a public health problem based on clinical signs and symptoms noted in Table 1 are well established. Prevalences based on subclinical biological indicators in Table 2 are state-of-the-art estimates. Because no one subclinical indicator is considered definitive in determining vitamin A status, the consultation recommended that before concluding that an important public health problem exists, at least two indicators should be prevalent below the cut-off for a deficiency, or that any one be strongly supported by a composite of several indirect demographic and ecological risk factors.

49. The conclusion that a public health problem exists automatically should trigger intervention. For VAD, the appropriate intervention strategy is likely to involve a mix of measures that will result in improved vitamin A status. The composition of the intervention mix may be influenced by the relative level of public health importance assigned to the problem, i.e. mild, moderate or severe. A severe problem usually requires interventions to concurrently address short-term health needs through the use of a vitamin A supplement and long-term permanent solutions to the problem through food-based approaches. A mild problem may be resolved without use of supplements through nutrition and social marketing interventions to increase dietary intake of vitamin A-containing foods by vulnerable groups.

50. The prevalence of values below cut-off for the subclinical biological indicators discussed in the remainder of the report are ranked from a global perspective as mild, moderate and severe levels of public health importance. Individual country, area, or population-based situations to trigger interventions may call for different rankings. This is a decision that local or national authorities should make.

PART III. BIOLOGICAL INDICATORS OF VITAMIN A STATUS

3.1 Clinical indicators

Xerophthalmia

51. Clinically obvious eye signs of vitamin A deficiency, i.e. Bitot's spot, corneal xerosis, keratomalacia and corneal scars, are well-established indicators of severe VAD. They are rare events in most population surveys, however, and require a large sample size to establish their prevalence. These signs are reviewed in detail in other WHO documents.¹⁵

52. For the sake of completeness, a summary of the classification of xerophthalmia, and the criteria for assessing in a community a significant public health problem in children under 6 years of age, are presented in Table 7. These criteria, which are unchanged from those presented in earlier documentation, are appropriate for application in communities where blinding malnutrition is observed. It should be noted that night blindness (XN) is part of the traditional classification of xerophthalmia symptoms and signs even though, unlike other signs of xerophthalmia, it is not readily apparent. At present, from a practical standpoint, night blindness in children 24-71 months of age, can be assessed only by taking a history from mothers. However, this may not be a reliable indicator below 24 months of age, or in some cultures even in the youngest of the age span indicated, since very young children are not particularly mobile after dark, with the result that their night blindness may go unnoticed. (Night blindness will be reviewed in greater detail later in this report together with other measures of subclinical VAD.)

¹⁵ (a) *Control of vitamin A deficiency and xerophthalmia*. Technical Report Series 672, World Health Organization, Geneva, 1982;

(b) *Vitamin A supplements. A guide to their use in the treatment and prevention of vitamin A deficiency and xerophthalmia*. WHO/UNICEF/VACG. World Health Organization, Geneva, 1988 (revised version in press, 1996);

(c) Sommer, A. *Vitamin A deficiency and its consequences: a field guide to their detection and control*. 3rd edition. WHO, Geneva, 1994.

Table 7

Classification of xerophthalmia, minimum prevalence criteria and minimum sample sizes for defining clinical VAD of public health importance^a in a community of children under 6 years of age.

	Indicator ^b	Minimum prevalence	Minimum ^c sample size
XN	Night blindness	1.0%	1522
X1A	Conjunctival xerosis	not used	-
X1B	Bitot's spot	0.5%	3058
X2	Corneal xerosis	0.01%	153 640
X3A	Corneal ulceration/keratomalacia < 1/3 corneal surface		
X3B	Corneal ulceration/keratomalacia ≥ 1/3 corneal surface		
XS	Corneal scar	0.05%	30 718
XF	Xerophthalmic fundus	not used	-

^a The minimum prevalence criteria for assessing a public health problem was not reviewed at the consultation. New evidence of preventing mortality and severe morbidities by improving the vitamin A status in populations with xerophthalmia suggests that there is an important public health problem when any xerophthalmia is noted.¹⁶

^b The biochemical indicator, serum retinol <0.35 µmol/l at a minimum prevalence ≥5% is strong corroborative evidence of any clinical criteria met for defining severe VAD.

^c Sample size is calculated for a relative precision of 50% at the 95% confidence level.

53. Clinical signs of xerophthalmia are inadequate for assessing the prevalence of non-clinically observable deficiency, i.e. depletion of vitamin A stores to the level where important functional consequences for health are likely to occur. Unfortunately, adequate quantitative data are not available on the critical body-store level below which functions are impaired. Nevertheless, the remainder of this report concerns those indicators, including night blindness, used to assess the spectrum of vitamin A deficiency which is not clinically apparent.

¹⁶ Beaton G., et al. Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries. ACC/SCN State-of-the-Art Series, Nutrition Policy Discussion Paper No. 13, December 1993.

3.2 Subclinical indicators

3.2.1 Functional indicator

Night blindness (XN)

54. Biological features. Night blindness, or poor adaptation to the dark, is the first functional manifestation of VAD that potentially can be measured. It is the result of a reduced rate of regeneration of rhodopsin in the outer segments of the rods following exposure to light, i.e. delayed dark adaptation (DA). There are instruments for measuring DA objectively in institutional settings but there is no objective field-applicable instrument yet available for measuring delayed DA in children below about 4 years of age. Under field survey conditions, in some circumstances symptoms of night blindness can be learned about from mothers of children who are at least 24 months of age. Below this age, children who are malnourished are not usually very mobile at dusk and after dark, so that night blindness could tend to go unnoticed. Another explanation could be that where breast-feeding typically continues up to 24 months of age, night blindness rarely occurs until after weaning. For populations above 4 years of age, in addition to interview techniques, several objective assessment procedures are presently being developed to measure pupillary and visual thresholds¹⁷ and vision restoration time (VRT).¹⁸ However, until these procedures are standardized and reference data for interpreting them are available, night blindness should be assessed by history.

55. Acceptability. Assessment of night blindness when using an interview technique does not present problems of acceptability.

56. Technical feasibility. Reliable assessment of night blindness by interview requires that there be a specific local word descriptive of the symptoms characteristic of this condition and that it be specific to vitamin A deficiency. There is always the risk of a high number of positive responses if the respondent believes that such a response may result in some beneficial treatment or therapy. More reliable responses may be elicited if a series of questions are asked to focus attention on visual acuity under different lighting conditions, i.e. during the day, evening and night, which are related to vitamin A deficiency. Also, since words for night blindness have multiple meanings in some cultures, this may lead to misclassification. It is important to field-test the reliability of local words prior to undertaking a large-scale survey. To make history of night blindness a more reliable indicator, it is also important to standardize the procedure for data collection, i.e. train interviewers and standardize their approach to asking questions.

57. Uses and application. Night blindness can be a useful tool for assessing community vitamin A status and highlighting those areas where the risk of health consequences from VAD among children may be expected. Prevalence of night blindness can be used as a mapping tool for identifying areas where interventions should be targeted. At the same time, however, night blindness tends not to be

¹⁷Congdon, N, et al. Pupillary and visual thresholds in young children as an index of population vitamin A status. *American Journal of Clinical Nutrition*, 1995, 61:1076-82.

¹⁸ Underwood, BA and Olson, JA (eds). *A brief guide to current methods of assessing vitamin A status*. A report of the International Vitamin A Consultative Group (IVACG). The Nutrition Foundation, Inc., Washington, D.C., 1993.

very discriminating, and is susceptible to error due to the indicator's subjective nature. Nonetheless, where it is a well-recognized symptom, it can be used as an assessment indicator and for surveillance of an intervention programme's effectiveness. Moreover, night blindness is an indicator that communities can grasp as a marker of VAD and which they can use to gauge an improvement resulting from an intervention. As such, it has the potential to increase awareness in communities of the importance of the problem and can be of great use for advocacy in helping to demonstrate where the problem of VAD is still evident and where it has disappeared, and in creating public demand for an intervention programme.

58. A history of night blindness is an important indicator to be applied both to pregnant and lactating women and preschool-aged children. However, few representative population-based surveys have been done among adult female populations.

59. Confounders. A significant age trend is observed in the prevalence of night blindness obtained by interview, i.e. little night blindness is detected in very young children for the reasons already noted, while rates increase in children from 2-6 years of age and older. Therefore, to use this indicator of VAD the age group for which the indicator is applicable should be standardized to a representative sample of preschool children between 24-71 months of age, school-aged children 6-14 years of age, and adults 15 years of age and above. Night blindness can be sensitive to seasonal variations in vitamin A status, and that associated with pregnancy may respond, without intervention, in the postpartum period. Also, night blindness is less specific in adults and occasionally has been confused with the rare hereditary eye disease *retinitis pigmentosa* where night blindness is reported from school ages through adulthood.

60. Performance. In some circumstances the classification of night blindness by interview has been improved by establishing an algorithm:

- (1) Does your child have any problem seeing in the daytime?
- (2) Does your child have any problem seeing at nighttime?
- (3) If (2) = yes, is this problem different from other children in your community?
(Note: this question is particularly appropriate where VAD is not very prevalent)
- (4) Does your child have night blindness (use local term that describes the symptom)?

This algorithm may increase the specificity and reduce the error of classification solely on the basis of familiarity with the term. The use of focus group discussions¹⁹ in different situations may be useful in identifying local terms for night blindness and the specificity with which they are useful for identifying VAD.

¹⁹ Dawson, S, Manderson, L & Tallo, VS *A manual for the use of focus groups: Methods for social research in disease*. WHO Social and Economic Research (SER), UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). International Nutrition Foundation for Developing Countries (INFDC), Boston, MA, USA, 1993.

61. Although a good correlation between night blindness and other measures of VAD among preschool children is reported in communities, the correspondence is not as good at the level of the individual. To establish more firmly the sensitivity and specificity of night blindness, there is a need for further research to clarify the relationship between night blindness as assessed through interview and different objective measures of DA or VRT and other indicators of vitamin A status.

62. Interpretation: Suggested interpretations of prevalences of night blindness to identify a public health problem are given in Table 8.

Table 8

Prevalence of night blindness in children 24-71 months of age
and minimum sample sizes for identifying a VAD public health problem

Level of importance as a public health problem	Prevalence	Minimum Sample Size ^a	
		20%	50%
Mild	>0 - <1%	—	—
Moderate	≥1% - <5%	9508	1522
Severe	≥5%	1825	292

^a Minimum sample size for anticipated prevalence with relative precision of 20% and 50% at the 95% confidence level. See annex 4.

3.2.2 Biochemical indicators

Serum retinol²⁰

63. Biological features. Vitamin A circulates in blood as retinol bound to its specific carrier protein, retinol-binding protein (RBP), which in turn is bound to transthyretin, a larger carrier protein that also transports thyroxine. Some retinyl ester is found, but at low concentrations except after vitamin A supplements or a meal rich in the vitamin have been ingested. The level of retinol in the blood is under homeostatic control over a broad range of body stores and reflects body stores only when these are very low or very high. Thus, serum concentration is not a reliable indicator to use for diagnosing vitamin A deficiency in individuals. Among populations, however, a frequency distribution of serum retinol concentrations can be very informative.

²⁰ Plasma can be used interchangeably with serum.

64. A disadvantage in using serum retinol concentration as an indicator of vitamin A status is that retinol concentrations are decreased by acute and underlying chronic infections.²¹ Given the seasonality in disease patterns, shifts in serum retinol distributions may occur that are unrelated to availability of vitamin A-rich foods. The extent to which disease patterns affect serum retinol distributions in populations is not fully understood. This is a reason for cautious interpretation when comparing serum retinol distributions as a specific indicator of vitamin A status among different populations. However, changes over time in serum retinol distributions within a population can be interpreted with greater confidence.

65. Acceptability. To the extent that blood collection by either venepuncture or finger prick are acceptable, measurement of retinol in serum can be used to characterize a population's vitamin A status. In some cultures it is very difficult to obtain blood samples from children unless done in a clinic or in association with some economic or medical benefit, e.g. a free meal or deworming. Careful consideration should be given to the risks of obtaining blood in populations where there is a high risk of HIV infection and all necessary precautions should be taken.²²

66. Technical feasibility. Collection of blood by venepuncture or finger prick are well-established procedures.²³ Blood should be protected from light and chilled (not frozen) until centrifuged. Separation of the serum by centrifugation should be accomplished after clotting, preferably within 12 hours. The serum should be stored protected from light, oxygen and desiccation, and frozen until analysed. Unless these minimal conditions are possible under field conditions, reliable measurements can not be expected. Currently the reliability of techniques using filter paper as the collection, storage and transport vehicle are under investigation but are not yet at the stage where they can be recommended for routine use.

67. Retinol can be determined in serum by high pressure liquid chromatography (HPLC), or by fluorescence or UV spectrophotometry.²⁴ Choice of analytical method will depend on the resources available to obtain the necessary reagents of high quality and purity and to maintain the analytical instrument. HPLC is the method of choice because of its high specificity and sensitivity, but it is also the most expensive and difficult to maintain under the conditions prevailing in many laboratories in developing countries. Fluorometric methods are sensitive, quick and relatively inexpensive, but the laboratory must be able to maintain conditions that avoid interference from contaminating fluorescing substances. Spectrophotometric methods are the least expensive to use but also the least sensitive. Both retinol and retinyl esters can be reliably measured individually by HPLC. In contrast, spectrophotometric, fluorometric and immunoassay techniques generally measure total serum vitamin

²¹ Filteau SM et al. Influence of morbidity on serum retinol of children in a community-based study in northern Ghana. *American Journal of Nutrition*, 1993, 58:192-7.

²² Sommer A. *Vitamin A deficiency and its consequences: a field guide to their detection and control*. 3rd edition, WHO, Geneva, 1994.

²³ *Biochemical methodology for the assessment of vitamin A status*. A report of the International Vitamin A Consultative Group (IVACG). The Nutrition Foundation, Washington, D.C., 1982.

²⁴ Furr HC, Barua AB, Olson JA. Analytic methods. In: Sporn MB, Roberts AB, Goodman DS (eds) *The Retinoids: Biology, chemistry, and medicine*. 2nd edition. Raven Press, NY, 1993.

A. Each of these methodologies requires well-trained technicians capable of maintaining strict quality control procedures. Colorimetric methods are not recommended.

68. Some investigators consider that current data suggest that RBP in serum may be a suitable alternative to serum retinol. However, before being recommended for use in surveys, more work is required to confirm its reliability in populations with varying degrees of PEM and acute and chronic infections. RBP can be measured by ELISA methods, but reliable ELISA techniques are not yet commercially available. Although RBP can be measured by immunoassay techniques, the only commercially available procedures are expensive. Procedures under development use a blood spot collected on filter-paper for analysis of either RBP or retinol. If the blood spot procedure proves reliable, it will allow data from developing countries to be collected more readily than is now possible. The filter-paper collection of blood will also permit assessment of the status of other micronutrients such as iron (ferritin) and iodine (TSH) using the same blood spot.

69. Cost. Cost depends on the analytic method used and ranges from relatively high based on HPLC, to relatively low based on fluorescence and UV spectrophotometry. The HPLC system should have at least a manual injector, a pump, a single wave-length detector, and a chart recorder. While such a simple system may cost as little as US\$15 000, more sophisticated equipment including an automated injector, system controller, pump, variable wave-length detector, and an integrator that simplifies the procedure can cost US\$40 000 or more.

70. The column used for HPLC can be any type of C-18 reversed-phase column.²⁵ A guard column is highly recommended because serum extracts contain considerable concentrated lipid. The guard column, packed with material similar to the column, helps to filter the extract, thereby protecting the column and increasing its useful life. The average cost of a suitable column is US\$500 and of a guard column is US\$75.

71. A variety of solvent systems have been used; the simplest and most economical is a methanol:water mixture. The elution time of retinol, other retinoids and internal standards is dependent on the ratio of methanol to water.

72. Spectrophotometric techniques that use a high-quality recording UV spectrophotometer are best for obtaining maximum sensitivity, and if only a small blood sample (e.g. from a finger prick) is available, the spectrophotometer must be adapted to microanalytical procedures.²⁶ The cost of a suitable UV spectrophotometer varies from US\$5 000 to US\$15 000.

73. Performance. Serum retinol gives a reliable estimate of vitamin A stores only at the extremes because of the homeostatic control mechanisms mentioned above. However, population distribution curves, and proportions of individuals below selected cut-off values, can be useful in characterizing the

²⁵ Furr HC, Tanumihardjo SA, Olson JA. *Training manual for assessing vitamin A status by use of the modified relative dose response and the relative dose response assays*. Dept Biochem & Biophys, Iowa State University, Ames, Iowa, USA, 1992.

²⁶(a) Bessey OA, et al. The determination of vitamin A and carotene in small quantities of blood serum. *Journal of Biological Chemistry*, 1946, 166:177;

(b) Araujo CRC, Flores H. Improved spectrophotometric vitamin A assay. *Clinical Chemistry*, 1978, 24:386.

likely vitamin-A status of a population, especially in areas of VAD, and can also be useful in evaluating changing conditions, e.g. response to an intervention programme.

74. The specificity of serum values can be confounded because RBP is an acute phase protein. It can be profoundly affected by febrile and afebrile infections, even in individuals with relatively normal body stores of vitamin A.²⁷ Thus, acute and underlying chronic infections can confuse specificity in interpreting serum values when cut-off points are used. Furthermore, RBP has a short half-life that may limit synthesis in the presence of PEM, and hence hinder the mobilization of retinol from body stores. In addition, normal serum retinol values change with age, increasing from lower levels at birth to adult levels after puberty.²⁸ This age relationship should be considered when evaluating serum retinol data from infants and young children. Serum distribution curves do not correlate closely with the prevalence of clinical eye signs and not always with the prevalence of other indicators of VAD such as abnormal impression cytology or dose-response measures, or current dietary intake, although these different indicators tend to categorize populations similarly.

75. Interpretation. In the past, serum levels of vitamin A of $<0.35 \mu\text{mol/l}$ and $<0.70 \mu\text{mol/l}$ were used to describe populations with, respectively, deficient and low vitamin A status.²⁹ For the purposes of this consultation, deficiency was defined as occurring where tissues are depleted to a level of functional significance, even if this is not clinically evident. Presumably this occurs when blood levels are below homeostatic set-points that respond to improvement in vitamin A status. There is no direct evidence of the serum cut-off value where functional consequences, morbidity/mortality effects, begin to occur. For that reason, and for the sake of consistency, the cut-off value of $\leq 0.70 \mu\text{mol/l}$ has been retained to indicate a low vitamin A status. However, the prevalence level designating an important public health problem is lowered to 10% from the previously used 15%.³⁰

-
- ²⁷ (a) Reddy V et al. Relationship between measles, malnutrition and blindness: a prospective study in Indian children. *American Journal of Clinical Nutrition*, 1985, 44:924-30;
(b) Filteau SM et al. Influence of morbidity on serum retinol of children in a community-based study in northern Ghana. *American Journal of Clinical Nutrition*, 1993, 58:192-7;
(c) Louw JA, et al. Blood vitamin concentrations during the acute-phase response. *Critical Care Medicine*, 1992, 20:934-41;
(d) Butler JC et al. Measles severity and serum retinol (vitamin A) concentration among children in the United States. *Pediatrics*, 1993, 91:1176-81.

²⁸ Lewis CJ et al. Relationship between age and serum vitamin A in children aged 4-11 y. *American Journal of Nutrition*, 1990, 52:353-60.

²⁹ Arroyave G et al. *Methodologies for monitoring and evaluating vitamin A deficiency intervention programs*. A report of the International Vitamin A Consultative Group (IVACG). The Nutrition Foundation, Washington, D.C., 1989.

³⁰ *Hypovitaminosis A in the Americas*. Report of a PAHO Technical Group Meeting. Scientific Publication No. 198, Pan American Health Organization, Washington, D.C., 1970.

Table 9

Prevalence of serum values of vitamin A $\leq 0.70 \mu\text{mol/l}$ in children ≥ 1 year
and minimum sample sizes for identifying a VAD public health problem

Level of importance as a public health problem	Prevalence	Minimum Sample Size ^a	
		20%	50%
Mild	$\geq 2 - \leq 10\%$	—	—
Moderate	$> 10\% - < 20\%$	865	139
Severe	$\geq 20\%$	385	62

^a Minimum sample size for anticipated prevalence with relative precision of 20% and 50% at the 95% confidence level. See Annex 4.

76. More information is obtained when the entire distribution of serum retinol is examined in increments equivalent to $0.35 \mu\text{mol/l}$ and extending to at least $1.40 \mu\text{mol/l}$. Limited studies in some environments have shown that fewer than 5% of child populations with adequate vitamin A status (i.e. no evidence by dose-response tests of inadequate liver stores) have values less than $1.05 \mu\text{mol/l}$.³¹

Breast milk vitamin A concentration

77. Biological features. Breast-milk vitamin A concentration is a unique indicator of vitamin A status because it provides information about the vitamin A status of both the mother and the breast-fed infant.³² The mother's secretion of vitamin A into milk is directly related to her vitamin A status, at least when her vitamin A status is inadequate. All infants are born with low stores of vitamin A and depend on vitamin A-rich early breast milk, and subsequent concentrations in mature breast milk, to accumulate and maintain adequate stores until complementary foods provide significant additional amounts of vitamin A in keeping with the growing child's increasing requirements. Thus, the concentration of vitamin A in breast milk also serves as an indicator of an infant's likely vitamin A status.

78. Milk vitamin-A content is very high in colostrum (the milk secreted in the first 4-6 days postpartum), and remains high in transitional milk (days 7-21 postpartum). Vitamin-A concentrations stabilize in mature milk (after about day 21 postpartum), and thus milk concentrations after the first postpartum month are the most useful for estimating the vitamin A status of mother and infant.

79. Most of the vitamin A in breast milk is in the form of retinyl palmitate in the milk fat. During a feed, the fat content of milk is quite variable for an individual mother, the most important source of variation being from the first milk expressed from a full breast which is lowest in vitamin A, to the last

³¹ Flores H, et al. Serum vitamin A distribution curve for children aged 2-6 y known to have adequate vitamin A status: a reference population. *American Journal of Clinical Nutrition*, 1991, 54:707-11.

³² Stolzhus RJ, Underwood BA. Breastmilk vitamin A as an indicator to assess vitamin A status of women and infants. *WHO Bulletin*, 1995, 73:703-711.

milk expressed which has the highest content. This variation in milk vitamin-A concentration is unrelated to the mother's vitamin A status and must be accounted for when using breast milk as an indicator to assess vitamin A status of individuals. However, for the purpose of assessing vitamin A status of a population, assuming that samples are collected throughout the day and at varied periods following the last feed, it is not necessary to account for the sampling variation in milk fat because this variation will be randomly distributed among all samples provided that the collection period is randomly distributed. Where random sampling throughout the day is not possible, milk vitamin A values should be expressed relative to fat concentrations.

80. **Acceptability.** Collection of breast-milk samples by female health workers is generally acceptable even in traditional cultures. Acceptability is highest if staff, in addition to training in how to collect them, receive training in how to address mothers' potential concerns about giving milk samples. Mothers should be assured that giving a sample of their milk will not reduce the amount of milk that their baby will receive, but rather that the breast will be stimulated to produce even more when milk is removed. Health workers and field staff should be assured that taking a sample of a mother's milk places no particular strain on the mother.

81. Because mothers in most communities care about the quality of the milk they feed their infants, the use of breast milk vitamin-A concentrations to assess vitamin A status may be more understandable than other indicators of vitamin A status, e.g. blood-drawing. The well-being of infants as a concern common to health professionals and mothers alike is an advantage in enlisting cooperation at the survey stage, and potentially in evaluating interventions to improve maternal—and thereby infant—vitamin A status.

82. **Technical feasibility.** Milk samples should be collected from mothers 1-8 months postpartum, which means that colostrum and transitional milk are avoided. During this period, breast milk proximate composition is relatively stable³³ and is likely to provide the major source of dietary vitamin A for the infant, with complementary foods contributing little if any. When interpreted on a population basis, it is not necessary to control the time of day of sample collection or the time since the infant was last breast-fed (i.e. how "full" the breast is). The vitamin A concentration in milk samples varies according to these factors; "capturing" this variability in a survey will help to ensure that survey values represent the full variation in the milk that infants ingest.

83. If it is not possible to assure a random distribution in the time period for collection of breast-milk, fat can be determined by a simple "crematocrit" micro-method³⁴ that has been field-tested.³⁵ About 75 μ l of well-mixed breast milk is drawn into capillary tubes, which are then sealed at one end and spun in a haematocrit centrifuge. The length of the cream layer is measured and the amount of

³³ *The quantity and quality of breast milk.* World Health Organization, Geneva, 1985.

³⁴ Lucas A, Gibbs JAH, Lyster RLJ. Creamatocrit: simple clinical technique for estimating fat concentration and energy value of human milk. *British Medical Journal*, 1987, 22:1019-20.

³⁵ Prentice A, Prentice AM, Whitehead RG. Breast-milk fat concentrations of rural African women. I. Short-term variations within individuals. *British Journal of Nutrition*, 1981, 45:483-94.

fat determined by reference to a standard curve of fat concentrations established by a chemical method.³⁶

84. Milk samples can be obtained by manual expression or by using a simple breast pump, whichever is easiest and most comfortable for mother and staff. If a mother has difficulty expressing milk, allowing her infant to suckle from one breast will facilitate milk "let down", thereby aiding expression from the other. A sample from only one breast is sufficient. Samples of approximately 5 ml, which must be protected from light, should be collected into tubes or vials with air-tight caps. This condition can be satisfied by using amber or yellow tubes, or by covering clear tubes with foil.

85. As soon as possible after the sample is collected, aliquots of milk in the precise volume required for analysis should be removed to separate vials for longer-term storage. Precise sampling at the time of collection into vials that will be used later for analysis prevents difficulties in attaining uniform mixing after prolonged storage in a freezer. To remove aliquots, the milk sample should be homogenized, i.e. the cream should be well-dispersed throughout the milk. Since this is most easily achieved by gently swirling the sample just after it has been removed from the breast, the ideal procedure is to aliquot the milk samples at the time of collection. If this is not possible, milk samples may be carried on ice to a field station or laboratory to be aliquoted. If milk samples have been chilled, i.e. carried on ice, they should be re-homogenized before aliquots are removed. To do this, allow the sample to come to room temperature protected from light, then swirl the sample gently for about 30 seconds before taking aliquots. The pre-measured milk in the vials that will later be used for analysis can be stored at -20⁰ C until analysed.³⁷

86. The concentration of retinol in milk can be determined after saponification by HPLC, a spectrophotometric method, or a fluorometric method similar to those used to determine serum retinol. As with all laboratory analyses, it is crucial that appropriate standards are used and that laboratory quality control procedures are instituted.

87. Cost. Cost will depend on the analytic procedure chosen and will be similar to that for determining serum retinol. (See paragraphs 61-64)

88. Performance. For assessment, the sensitivity and specificity of the breast milk's vitamin-A concentration for identifying VAD in individual infants are not high; the sum of sensitivity and specificity is approximately 130% using a variety of cut-off values. Nonetheless, the association between vitamin A in breast milk and other maternal and infant vitamin A status indicators is sufficiently strong that it is a useful indicator at the population level.

89. The distribution of breast milk vitamin A-levels and the prevalence below selected cut-offs provides information about the risk of mild-to-moderate VAD in infants. However, even when VAD is endemic in a community, breast milk usually provides enough vitamin A to prevent clinical deficiency

³⁶ Ferris AM, Jensen RG Lipids in human milk: A review. 1: Sampling, determination, and content. *Journal of Pediatric Gastroenterology and Nutrition*, 1981, 45:483-94.

³⁷ Stoltzfus, R.J. et al. High-dose vitamin A supplementation of breast-feeding Indonesian mothers: effects on the vitamin A status of mother and infant. *Journal of Nutrition*, 1993, 123:666-75.

in infants if breast milk is the primary source of calories. Furthermore, the morbidity-sparing effect of breast-feeding reduces the negative impact of illnesses on an infant's vitamin A status. Thus, clinical vitamin A deficiency is extremely rare among breast-fed infants, and milk vitamin A concentration is not a sensitive indicator to predict risk of clinical vitamin A deficiency.

90. Interpretation. In vitamin A-sufficient populations, average breast milk vitamin A concentrations range from 1.75-2.45 $\mu\text{mol/l}$, whereas in a vitamin A-deficient population, average values are below 1.4 $\mu\text{mol/l}$.³⁸ A cut-off of $\leq 1.05 \mu\text{mol/l}$, or $\leq 8 \mu\text{g/g}$ milk fat, is selected based on considerations of the dietary vitamin A requirement of infants and its usefulness in monitoring changes in the vitamin A status of mothers.

91. For an infant who is exclusively, or almost exclusively, breast-fed from 0-6 months of age, and partially breast-fed from 6-12 months of age, a milk concentration of at least 1.05 $\mu\text{mol/l}$ provides enough vitamin A to meet metabolic needs but allows for little or no liver storage of vitamin A. This cut-off point thus represents the minimum concentration required to prevent subclinical deficiency in the first 6 months of life.³⁹ These theoretical calculations are supported by the observation that among a population of mothers in Central Java, Indonesia, the prevalence of breast milk vitamin A concentrations below 1.05 $\mu\text{mol/l}$ was practically identical to the prevalence of positive RDR tests (an indication of inadequate vitamin A stores) among infants at 6 months of age.³⁷ Additional confirmation of this cut-off for breast milk adequacy with vitamin A status indicators is needed in other populations.

Table 10

Prevalence of breast-milk values $\leq 1.05 \mu\text{mol/l}$ ($\leq 8 \mu\text{g/g}$ milk fat) in a population of lactating women and minimum sample sizes for identifying a VAD public health problem

Level of importance as a public health problem	Prevalence	Minimum Sample Size ^a	
		20%	50%
Mild	<10%	—	—
Moderate	$\geq 10 - <25\%$	865	139
Severe	$\geq 25\%$	289	47

^a Minimum sample size for anticipated prevalence with relative precision of 20% and 50% at the 95% confidence level. See annex 4.

³⁸ (a) The quantity and quality of breast milk. World Health Organization, Geneva, 1985;

(b) Newman V. Vitamin A and breastfeeding: a comparison of data from developed and developing countries. Wellstart International, San Diego, CA, 1992.

³⁹ Underwood BA. Maternal vitamin A status and its importance in infancy and early childhood. *American Journal of Clinical Nutrition*, 1994, 59 (suppl): 517S-24S.

92. Other surveillance uses. The prevalence of breast milk vitamin A concentrations below 1.05 $\mu\text{mol/l}$ is a sensitive measure of change in vitamin A status following a related intervention. This has been reported in evaluating high-dose supplementation of women in the postpartum period³⁷ and in evaluating sugar⁴⁰ and monosodium glutamate (MSG) vitamin A fortification interventions. Surveillance of breast milk vitamin A concentration is thus a useful tool for identifying high-risk areas/populations, evaluating vitamin A interventions, and monitoring changes in the vitamin A status of communities.

Relative dose response tests (RDR and MRDR)

93. Biological features. Even if the synthesis of retinol binding protein (apo-RBP) in the liver is not controlled by vitamin A status, its release still depends on the availability of vitamin A from dietary sources or endogenous body stores. Therefore, at times of reduced availability of retinol, e.g. during periods of fasting or depletion of body stores, apo-RBP accumulates in the liver. Although retinol (R) is the preferred ligand for apo-RBP, 3,4-didehydroretinol (variably referred to as dehydroretinol, vitamin A₂, or DR) is a suitable signal for RBP release. R or DR binds to apo-RBP in the liver and the holo-RBP complex is released. Holo-RBP circulates in the plasma bound to another carrier protein, transthyretin. After a suitable oral dose, R or DR should appear in the plasma in significant amounts above baseline levels only when endogenous liver retinol concentrations are inadequate. Current data suggest that this occurs when liver reserves of vitamin A are below 0.07 $\mu\text{mol/g}$. DR is a naturally occurring form of vitamin A that is found predominantly in fresh-water fish, but also to a small extent in mammalian, including human, tissues. DR has approximately 40% of the biological activity of retinol.

94. Acceptability. Blood-taking has to be culturally acceptable to the populations whose vitamin A status is to be assessed. Suitable specimens may be obtained by finger prick or venepuncture. Because two samples are required with a 5-hour interval for the RDR, acceptability of blood-taking is generally lower than for the MRDR, which requires but a single specimen after a similar post-dosing wait interval. For the MRDR, however, the dose can be administered at home, thereby requiring the subject to come only once for blood collection 4-6 hours later.

95. Technical feasibility: RDR.⁴² After obtaining a fasting blood sample, a dose of 450-1000 μg retinyl (R) ester (palmitate or acetate) in an oily solution is given either by direct administration from a calibrated dispenser onto the tongue or absorbed on a suitable food vehicle such as a cracker or piece of bread. Following the dose a simple breakfast is given which should contain some fat to aid absorption, but little vitamin A. Water-miscible preparations dissolved in fruit juice can also be used. Five hours after dosing, a second blood sample is obtained. Retinol in the fasting pre-dosing (A₀) and

⁴⁰ Arroyave, G. Vitamin A deficiency control in Central America. In: *Vitamin A Deficiency and Its Control*. JC Bauernfeind, ed. Academic Press, Inc., NY, 1986.

⁴¹ Muhilal, MA et al. MSG and vitamin A status: a controlled field trial. *American Journal of Clinical Nutrition*, 1988, 48:1265-70.

⁴² Underwood, BA. Methods for assessment of vitamin A status. *Journal of Nutrition*, 1990, 120:1459-63.

post-dosing (A_5) samples is determined by a suitable analytical technique that can include spectrophotometry, fluorometry or HPLC.

96. The RDR is calculated as a percentage in the following manner:

$$\text{RDR} = \frac{A_5 - A_0}{A_5} \times 100$$

97. Because UV spectrophotometry and fluorometry determine total serum vitamin A, when these analytical techniques are used, the dose given should be about 450 μg (not less) for children and about 600 μg for adults. If a higher dose is used it is possible that newly absorbed esters will remain in the circulation after 5 hours, and a high vitamin A value could either be due to a low liver store of vitamin A (retinol released from the liver after binding to apo-RBP), or to retinyl esters which are still circulating in the blood. On the other hand, retinyl esters can be distinguished from retinol when analysed by HPLC. It is the retinol level which should be measured, and feeding the higher dose level, i.e. 600-1000 μg , assures that absorption problems, e.g. those resulting from heavy enteric parasite load, are overcome. Within these limitations, the exact amount fed is not critical because the HPLC technique specifically measures the amount of the absorbed vitamin that passes through the liver, i.e. holo-RBP.

98. There are constraints in the use of the RDR as an indicator in field surveys. Because two blood samples are necessary within a 5-hour interval there may be compliance problems in obtaining the second sample from young children, and logistical problems in caring for them during the interval without disrupting survey activities. An effective procedure consists of offering a simple breakfast after obtaining the specimen, proceeding with a physical examination during the interval, and providing opportunities for health education (e.g. by games, dramas, puppet shows, cooking demonstrations, videos, etc.) and immediate feedback to the mother.

99. Technical feasibility: MRDR.⁴³ The MRDR assay involves giving children a single oral dose (100 $\mu\text{g}/\text{kg}$ body weight, or a 1.5 mg standard dose) of 3,4-didehydroretinyl acetate dissolved in an oil, and taking a single venous blood sample 4-6 hours later. A standard dose of 2.5 mg has been used in adult studies. After the serum is extracted with ethanol/hexane, R and DR in an aliquot are measured by HPLC. The monitoring wavelength of the detector is set at 350 nm, which optimizes the measurement of DR. Standards of both R and DR of known concentration are used to calibrate the HPLC system. A molar ratio of DR to R can thereby be calculated:

$$\text{MRDR} = \frac{\text{Serum DR concentration}}{\text{Serum R concentration}}$$

100. The amount of serum needed for analysis depends on several factors. Firstly, the limits of detection on the HPLC system must be determined. The typical range of DR concentrations found in serum from the MRDR test is 1 to 30 ng/ml. Typically, 0.5 to 2 ml blood samples are obtained

⁴³ Furr, HC. Tanumihardjo, S.A. and Olson, JA. *Training manual for assessing vitamin A status by use of the modified relative dose response and the relative dose response assays*. Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa, 1992.

under field conditions from children 1-6 years of age. A serum aliquot of 0.15 to 0.5 ml is usually satisfactory for detecting DR. Thus, sufficient blood should be collected to perform the analysis on duplicate samples. By employing the ratio of DR/R in a single serum sample, the variability of storage effects on vitamin A stability and of sample extraction efficiency are minimized. Another advantage is that prolonged waiting at the site can be avoided by giving children the dose in the morning at home and bringing them to the clinic or the survey site 4-6 hours later for sampling.

101. As with the RDR, there are some limitations in applying the MRDR procedure. Free DR is not stable once extracted from serum, and must therefore be carefully protected from light and analysed immediately by HPLC. Currently an important constraint is that DR is not available commercially and must either be synthesized⁴⁴ or isolated directly from fresh-water fish-liver oils.

102. Costs. The RDR can be done using any of the procedures for serum retinol analysis discussed earlier but MRDR requires HPLC instrumentation for the analysis to detect DR in the serum. Thus costs for instrumentation are those already discussed for serum retinol determinations in paragraphs 61-64. The cost for obtain DR from the current single supplier is about US\$2 000 for 0.5 g and US\$3 000 for 1 g.⁴⁵

103. Interpretation: RDR. A response $\geq 20\%$ is indicative of inadequate liver reserves (about $<0.07 \mu\text{mol/g}$ liver). Therefore, if the RDR value is $\geq 20\%$, the child is almost certainly vitamin A deficient. However, the relationship between the RDR and liver stores is not necessarily linear above or below the critical level, i.e. $\geq 20\%$. Among children with adequate vitamin A status, negative RDR values sometimes occur. To determine if a particular community is at risk, deficiency status can be assessed in a randomly sampled sub-population of the total population of preschool children for which serum-distribution curves are also randomly obtained. This helps to overcome problems associated with interpretation of serum-distribution curves in the range of about $0.52\text{--}1.05 \mu\text{mol/l}$ as a reflection of vitamin A status.⁴⁶ Thus, only a statistically adequate, representative number of children need be studied to make suitable public health judgements about a population's vitamin A status.⁴⁷ A public health problem of moderate or severe importance might be assumed to exist if $\geq 20\%$ of a population of preschool children show abnormal RDR ($\geq 20\%$) values.

⁴⁴ Tanumihardjo SA, Barua AB, Olson JA. Use of 3,4-didehydroretinol to assess vitamin A status in rats. *International Journal of Vitamin Nutrition Research*, 1987, 57:127-32.

⁴⁵ Available from Retinoid Services, Iowa State University, Ames, Iowa, USA following several months advanced notice to Dr Sherry Tanumihardjo (Tel: 515-294-2646; Fax: 515-294-4141; e-mail: sherry@iastate.edu).

⁴⁶ (a) Flores H, et al. Assessment of marginal vitamin A deficiency in Brazilian children using the relative dose response procedure. *American Journal Clinical Nutrition*, 1984, 40:1281-9;

(b) Wahed MA, et al. Comparison of the modified relative dose response (MRDR) and the relative dose response (RDR) in the assessment of vitamin A status in malnourished children. *American Journal of Clinical Nutrition*, 1995, 61:1253-6.

⁴⁷(a) Report on the prevalence of inadequate vitamin A nutriture in preschool children of North and Northeast Thailand, Nutrition Division, Dept. of Health, Ministry of Public Health and Institute of Nutrition, Mahidol University, Bangkok, Thailand, 1991.

(b) Sultanate of Oman, Ministry of Health, National Study on the prevalence of vitamin A deficiency (VAD) - Oman. *Community Health and Disease Surveillance Newsletter*, Vol. IV, No. 1, Jan-Mar, 1995.

104. Vitamin A supplements should be given to populations or clusters of children where a severe problem is detected by a high prevalence ($\geq 30\%$) of positive RDR values, and considered where prevalence is moderately severe ($\geq 20 - < 30\%$). When a suitable vitamin A-rich local food, or vitamin A-fortified food product, is available, consistent consumption will also reduce the prevalence of positive RDRs.⁴⁸ A food approach should be encouraged wherever feasible as a sustainable intervention for replacing supplements within a specified period.

105. Interpretation: MRDR. The ratio DR/R has been evaluated in a variety of population groups whose vitamin A status ranged from normal to abnormal, and its reproducibility evaluated.⁴⁹ Experience to date suggests that a moderate public health problem exists when $\geq 20 - < 30\%$ of a general population has ratios ≥ 0.06 , and a severe problem when $\geq 30\%$ of a population's preschool children are classified as deficient.

106. Table 11 summarizes the prevalence for determining if an important public health problem exists using either the RDR (cut-off for deficiency = $\geq 20\%$) or MRDR (cut-off for deficiency is DR/R ratio = ≥ 0.06).

Table 11

Prevalence of a positive RDR or MRDR and minimum sample sizes
for identifying a VAD public health problem

Level of importance as a public health problem	Prevalence RDR ($\geq 20\%$)	Prevalence MRDR (≥ 0.06)	Minimum Sample Size ^a	
			20%	50%
Mild	$< 20\%$	$< 20\%$	—	—
Moderate	$\geq 20 - < 30\%$	$\geq 20 - < 30\%$	385	62
Severe	$\geq 30\%$	$\geq 30\%$	225	36

^a Minimum sample size for anticipated prevalence with relative precision of 20% and 50% at the 95% confidence level. See annex 4.

⁴⁸ Mariath J, Lima M, Santos L. Vitamin A activity of Buriti (*Mauritia Vinifera Mart*) and its effectiveness in the treatment and prevention of xerophthalmia. *American Journal of Clinical Nutrition*, 1989, 49:849-53.

- ⁴⁹(a) Tanumihardjo SA, Koellner PG, Olson JA. 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*, 1990, 52:1064-7;
- (b) Tanumihardjo SA, et al. Vitamin A status in preschool-age Indonesian children as assessed by the modified relative-dose-response assay. *American Journal of Clinical Nutrition*, 1990, 52:1068-72;
- (c) Tanumihardjo SA, Olson JA. The reproducibility of the modified relative dose response (MRDR) assay in healthy individuals over time and its comparison with conjunctival impression cytology (CIC). *European Journal of Clinical Nutrition*, 1991, 45:407-11.
- (d) Tanumihardjo SA, et al. Vitamin A status of Indonesian children infected with *Ascaris lumbricoides* after dosing with vitamin A supplements and albendazole. *American Journal of Clinical Nutrition*, 1996;126:451-7.

Serum 30-day dose response test (+S30DR)⁵⁰

107. Biological features. The basic principles behind the +S30DR are similar on a population basis to that of the RDR and MRDR. Thus a population with a significant number of vitamin A-deficient individuals at baseline as measured by the distribution curve of serum levels will respond to improvement in vitamin A status in the population by a shift in the left portion of the distribution curve, which is demonstrable about 30-45 days (or longer depending on the type of programme) following the implementation of a vitamin A intervention programme. The approach has been applied most often to evaluate mega-dose supplementation programmes. The assumption is that, on a population basis, a significant number of individuals have vitamin A levels below what their homeostatic level would be if their vitamin A status were adequate. This indicator is most appropriate in monitoring the effectiveness of intervention programmes for improving vitamin A status in a specified recipient population or geographic area.

108. Acceptability. The acceptability of the procedure is dependent on the willingness of a population to provide blood samples. Because the procedure involves obtaining two blood samples from the same child about 30 days apart, it is more likely to be acceptable than obtaining blood twice from the same subject within about 5 hours, as required for the RDR. On the other hand, unlike the RDR or MRDR, both of which can be evaluated at the individual level, the +S30DR can be interpreted only at the population level.

109. Technical feasibility. The procedure is to obtain a fasting blood sample before, and 30-45 days after, introduction of a vitamin A intervention programme, such as providing mega-dose (200 000 IU) vitamin A supplements to a selected population, usually preschool-aged children. Blood samples are obtained from a randomly selected population at baseline, and repeated on the same population at 30-45 days post-dosing. A laboratory that is equipped to do vitamin A determination by any standard procedure providing reliable results is required. Colorimetry is not recommended.

110. Interpretation. Experience in using this procedure to evaluate the effectiveness of a community-based, periodic universal distribution of mega-doses of vitamin A in Northeast Brazil demonstrated a shift in the left portion of the population distribution curve in response to successive distribution rounds.⁵¹ Movement of the curve stabilized following the third distribution round. When this approach was validated against the RDR applied in a population sub-sample at the beginning of the programme, a sensitivity of 88% and a specificity of 70% were found.

111. The +S30DR is calculated as follows:
$$\frac{T_1 - T_0}{T_1} \times 100$$

⁵⁰ Flores H, et al. Frequency distributions of serum vitamin A levels in cross-sectional surveys and in surveys before and after vitamin A supplementation. In: Underwood BA & Olson JA (eds), *A brief guide to current methods of assessing vitamin A status*. A report of the International Vitamin A Consultative Group (IVACG). The Nutrition Foundation, Washington, D.C., 1993.

⁵¹ Flores H, et al. Serum vitamin A distribution curve for children aged 2-6 y known to have adequate vitamin A status: a reference population. *American Journal of Clinical Nutrition*, 1991, 54:707-11.

where T_1 is the serum vitamin A value 30-45 days after an intervention, and T_0 is the baseline level before the intervention. A cut-off of $\geq 20\%$ is used to identify a deficient individual at baseline. The prevalence of positive +S30DR to identify a public health problem is given in Table 12.

Table 12

Prevalence of positive +S30DR value in children 1-6 years of age
and minimum sample sizes for identifying a VAD public health problem

Level of importance as a public health problem	Prevalence	Minimum Sample Size ^a	
		20%	50%
Mild	<20%	—	—
Moderate	$\geq 20 - < 30\%$	385	62
Severe	$\geq 30\%$	225	36

^a Minimum sample size for anticipated prevalence with relative precision of 20% and 50% at the 95% confidence level. See annex 4.

112. The procedure identifies communities responsive to improvement in vitamin A status, which are those where vitamin A interventions are expected to provide effective health benefits. This approach is thus especially useful for monitoring change in status in an area in response to a programme. Because there is no need to retain children for 5 hours as with the RDR or MRDR tests, it is logistically simpler under field conditions.

3.2.3 Histological indicator

Impression cytology

113. Biological features. Vitamin A deficiency generally means that the integrity of epithelial cells is compromised. The presence of vitamin A allows stem cells to transform to mucin-secreting cells (goblet cells) while maintaining the columnar epithelial cells. By examining the morphology of epithelial cells obtained from the conjunctival surface on a piece of filter-paper, it is possible to assess whether changes have occurred that are associated with vitamin A deficiency. A normal impression of conjunctival cells will reveal sheets of small epithelial cells and an abundance of mucin-secreting goblet cells. In vitamin A deficiency, the epithelial cells are flattened and enlarged, and there is a marked reduction or absence of goblet cells.⁵²

114. Acceptability. Impression cytology generally has been well accepted in the field among children above 3 years of age. In some cultures, investigators have successfully obtained specimens

⁵² Natadisastra G, et al. Impression cytology for detection of vitamin A deficiency. *Archives of Ophthalmology*. 1989, 105:1224-8.

even in infants. Acceptance is improved if the procedure is explained and demonstrated before hand, and the subject is reassured that there will be no negative consequence associated with obtaining a specimen. When specimens are taken by hand, by applying a strip of filter-paper to the temporal portion of the bulbar conjunctiva, there is a high rate of compliance.⁵³ A disk applicator has been used which allows better targeting of specimen location, is quicker, and generally provides a better quality specimen that, in turn, corresponds more closely to serum retinol levels.⁵⁴ However, in some cultures the disk applicator has been well accepted, while in others it has caused concern among both subjects and mothers. The disk applicator may be more widely accepted in clinics, while the hand-held strip is more exportable into homes and, in general, appears less invasive.

115. Technical feasibility. Samples are obtained by applying a small strip of filter-paper of standard pore size to the lower temporal surface of the conjunctiva of one eye, followed by a separate strip applied to the other eye. This can be done by hand-touch or disk applicator contact of filter-paper with the conjunctiva. Practical experience is critical to obtaining good specimens by either technique. There is then a choice of two procedures for handling specimens.

116. ICT.⁵⁵ For impression cytology with transfer (ICT) the sample of cells adhering to the filter-paper is immediately transferred to a clean microscope slide and immersed for 20 minutes in a single staining solution that contains ethanol as a fixative. The slide is rinsed, allowed to dry, and stored without further processing until evaluated. Staining can be performed easily in the field as it requires exposure to a single staining solution only and there are no special storage requirements. However, clean slides and good practice are crucial to obtaining a good transfer from the filter-paper to the slide. There is no limit to the number of slides that can be processed daily by ICT. Practically speaking, 10-12 slides can be stained at once in a container. The stain for the ICT is inexpensive, readily available and quite stable. ICT samples are minimally affected by exposure from atmospheric moisture during storage and can be retained indefinitely without mounting.

117. CIC.⁵⁶ For conjunctival impression cytology (CIC) cells retained on filter paper are stored in a small bottle in a fixative of acetic acid, water and formaldehyde, which can be maintained at room temperature for months. There are several steps in the staining process, which are best accomplished at a base laboratory. After staining cells retained on the filter paper, the paper is sequentially dried in a series of 95% alcohol then 100% alcohol baths that totally dehydrate the paper with the stained cells. The dried paper is made transparent by soaking in xylene, and the transparent strip is mounted

⁵³ Kjolhede CL, et al. Conjunctival impression cytology: feasibility of a field trial to detect subclinical vitamin A deficiency. *American Journal of Clinical Nutrition*, 1989, 49:490-4.

⁵⁴ Keenum DG, et al. Assessment of vitamin A status by a disk applicator for conjunctival impression cytology. *Archives of Ophthalmology*, 1990, 108:1436-41.

⁵⁵(a) Luzeau, R., et al. Impression cytology with transfer: an easy method for detection of vitamin A deficiency. *International Journal of Vitamin Nutrition Research*, 1988, 58:166-70;

(b) Carlier C, et al. Conjunctival impression cytology with transfer as a field-applicable indicator of vitamin A status for mass screening. *International Journal of Epidemiology*, 1992, 21:373-80.

⁵⁶ Wittpenn JR et al. ICEPO Training manual. *Assessment of vitamin A status by impression cytology*. Dana Center for Preventive Ophthalmology, School of Hygiene and Public Health of The Johns Hopkins University, Baltimore, Maryland, USA, 1988.

using Permout. Care must be taken to avoid moisture during mounting and storage because this will render the strip opaque and unreadable. Achieving these conditions may be difficult in some environments. Samples that become opaque can be "rescued" before mounting by reimmersion in 100% ethanol. An experienced technician should be able to stain about 100-150 slides per day.

118. Technical requirements. Both CIC and ICT require slides, standard pore size filter- paper, and a simple light microscope. The difference is that for ICT a single staining solution is involved, while CIC requires more reagents and processing steps for fixing, staining and mounting specimens. Chemicals for CIC may be difficult to obtain and relatively expensive in some countries. On the other hand, CIC avoids difficulties that can occur with ICT in obtaining an efficient transfer of cells of high quality from filter paper to slide.

119. For standardization in obtaining specimens, at least 100 to 200 subjects need to be measured before a field worker is able to obtain highly reliable specimens for both ICT and CIC. Obtaining good samples is not easy, especially from young children, nor is perfecting the technique of transferring cells to slides without smearing for purposes of ICT. For either technique, staining should be done by someone who is well-trained. This is all the more important where the CIC procedure is concerned since more processing steps are involved. On the other hand, while specimen processing for ICT is simpler compared to CIC, the unreadable rate may be higher since the entire sample of cells is not transferred and available for evaluation.

120. Interpretation. Interpretation of slides is extremely important, and variability within and across studies has hindered this technique's widespread use. Standardization exercises, therefore, become critical in evaluating inter- and intra-observer variability. In Thailand, it has been demonstrated that following 3 days of intensive field-worker training, there was approximately 70-90% agreement with an experienced trainer. Training should be continued until Kappa values above at least 0.8 are achieved. It is important to have reference slides or photographs against which unknown slides can be compared. Possible ways of compensating for the subjective nature of this technique include having two readers and accepting as definitive only identical interpretations made by both. Discrepancies should be resolved by examining the slide further until a consensus is reached.

121. Cost. For ICT the cost, excluding labour, in Thailand was approximately US\$0.15 per specimen, while for CIC it was approximately US\$0.25-0.30 per specimen. Higher cost estimates for CIC, ranging from US\$0.80/person (about 1000-specimen volume) to US\$3.00/person (about 100-specimen volume), are reported from the USA. A standard quality light microscope is required (US\$2000-3000), although some improvement in readings may be realized with higher quality microscopes. The cost of the microscope represents the only capital outlay. There is some additional cost involved in the training and maintenance (salary) of a microscopist, which is a critical constraint. These costs can be significant, as the reading of each slide can take as long as 3-5 minutes, or 20 slides per hour, and no more than 100 slides per day. There are also maintenance costs associated with renewal of the reagents for CIC, some of which may become unstable.

122. Performance. To assess performance, a standard set of reference slides must be available for interpretation. For vitamin A there is no indicator that can be used as an unqualified standard to assess vitamin A status against which CIC or ICT specificity and sensitivity can be evaluated. Performance of CIC or ICT against various standards reveal very different interpretations as shown in Table 13. The

variability noted in the table is not surprising because each of the assessment indicators is measuring a different phenomenon in relation to vitamin A status. Probably the most logical comparison is between impression cytology and Bitot's spots because both indicators reflect different stages of altered cell morphology. The relatively low sensitivity and/or specificity relative to other non-ocular indicators of vitamin A status highlight the importance of using more than one indicator to assess subclinical vitamin A status to determine if an important public health problem exists.

Table 13

Comparison of the sensitivity and specificity of impression cytology against different indicators of VAD

Reference Standard	Sensitivity	Specificity	Reference
Bitot's spots	95	78	Wittpenn ⁵⁷
XN + Bitot's spots	93	94	Natadisastra ⁵⁸
RDR	26	88	Resnikoff ⁵⁹
RDR	23	80	Gadomski ⁶⁰
Serum ($\mu\text{mol/l}$)			
<0.35	75	48	Resnikoff ¹
<0.70	62	63	
<1.05	59	100	
< 0.70	26	81	Gadomski ²

¹ Used ICT

² UsedCIC

⁵⁷ Wittpenn JR et al. Detection of early xerophthalmia by impression cytology. *Archives of Ophthalmology*, 1986, 104:237-9.

⁵⁸ Natadisastra, G et al. Impression cytology for detection of vitamin A deficiency. *Archives of Ophthalmology*, 1987, 105:1224-8.

⁵⁹ Resnikoff S et al. Impression cytology with transfer in xerophthalmia and conjunctival diseases. *International Ophthalmology*, 1992, 16:445-51.

⁶⁰ Gadomski AM et al. Conjunctival impression cytology to detect subclinical vitamin A deficiency: comparison of CIC with biochemical assessments. *American Journal of Clinical Nutrition*, 1989, 49:495-500.

123. Interpretation. There is no generally accepted reference standard for the classification of vitamin A status on the basis of impression cytology readings. Currently three interpretation schemes are in use, one in which specimens are grouped as normal or abnormal based on the number of goblet cells or density of mucin spots,⁵⁷ and the other two with two additional intermediate gradations based on the quality of epithelial tissue.^{54,55} The consultation recommended that an abnormal classification be made only when both eyes are observed to be abnormal. If a slide is available for one eye only, the following recommendation pertains: if the slide is normal, then the child is classified as normal; if the slide is abnormal, it should be classified as uninterpretable and the individual excluded from the denominator for calculating prevalence rates since classification cannot be confirmed with a second impression.

124. The presence of mucin spots, which may be numerous in CIC specimens, has been difficult to interpret. With a defined training programme by experienced personnel, specimen interpretation skills are said to be readily transferable.⁶¹ Based on general experience where such special training is not available, the consultation recommended that interpretation of impression cytology specimens should be based exclusively on the morphology of the epithelial cells, and the presence and number of goblet cells. The prevalences suggested to identify a public health problem are shown in Table 14.

125. Limitations. There is a higher probability of an abnormal reading in populations with a high incidence of inflammatory ocular diseases like trachoma and conjunctivitis. This technique cannot be used alone to determine the prevalence of VAD in such populations, i.e. where the combined rate of conjunctivitis and trachoma is more than 5% of the population.⁵⁹

126. The experience of most field staff has been that good quality impressions are difficult to obtain from children under 24 months of age. The technique is more easily replicated in children 24-71 months of age and older. Interpretation in older children and adults may be less reliable, however, due to non-responsive Bitot's spots and other changes in the conjunctiva which are not specific to vitamin A deficiency, e.g. limbal vernal keratoconjunctivitis. Validation studies are encouraged before recommending impression cytology for use as an indicator of VAD for other age groups, in particular school children and pregnant and lactating women.⁶²

⁶¹ Keenun DG, et al. Inter-reader reproducibility of one hundred conjunctival impression cytology (CIC-A) specimens from Nepal. *Investigative Ophthalmology & Visual Science*, 1992, 33:(abstract 2583).

⁶² Stoltzfus RJ, et al. Conjunctival impression cytology as an indicator of vitamin A status in lactating Indonesian women. *American Journal of Nutrition*, 1993, 58:167-73.

Table 14

Prevalence of abnormal impression cytology in children 24-71 months of age and minimum sample size for identifying a VAD public health problem^a

Level of importance as a public health problem	% abnormal	Minimum Sample Size ^b	
		20%	50%
Mild	<20	-	-
Moderate	≥20 - <40	385	62
Severe	≥40	145	24

^a These cut-off points are provisional. To establish cut-off points, it is necessary to determine the rate of abnormal CIC or ICT in a healthy population in which there is no biochemical evidence of subclinical deficiency, and to compare this with the relative rate of abnormal impressions in populations with distinct degrees of VAD, as assessed by serum retinol, RDR or MRDR.

^b Minimum sample size for anticipated prevalence with relative precision of 20% and 50% at the 95% confidence level. See annex 4.

127. Uses. Given that impression cytology reflects the long-term effects of vitamin A deficiency in a population, i.e. is slow to respond to change in vitamin A status, it is important to combine this indicator with an indicator of the acute vitamin A situation. For this purpose, it may be possible to consider combining CIC or ICT with one or more additional other indicator(s) such as serum retinol, dietary intake, or RDR (MRDR).

PART IV. ECOLOGICAL AND RELATED INDICATORS ASSOCIATED WITH RISK OF VAD

4.1 Purpose and use of indirect indicators associated with VAD

128. Biological indicators, both clinical (xerophthalmia) and biochemical, are widely used to assess the prevalence and severity of VAD, and to evaluate the effectiveness of VAD control programmes. Another series of indicators of "risk" were discussed at the consultation, which are referred to as "ecological" and related indicators that have been associated with the "clustering" of VAD. Ecological and related indicators help to identify areas/populations where VAD is likely to be prevalent by focusing on factors that are responsible for, or contribute to, the problem's occurrence. Assessment of these risk indicators provides crucial information for planning and targeting VAD well-suited interventions, thus increasing the likelihood of programme effectiveness at the community level. These indirect indicators do not replace biological indicators and cannot be used alone for determining the vitamin A *status* of populations, or to define whether a population has a VAD problem of public health significance. However, a composite based on them can be used to corroborate biological criteria to determine if there is a public health problem.

129. The objectives for gathering information on indirect indicators are as follows:

- ◆ identifying areas/populations likely to be at high risk for vitamin A deficiency and where more specific biological investigations are needed;
- ◆ planning VAD control programmes where there is a problem;
- ◆ monitoring progress of vitamin A control programmes within a development framework;
- ◆ engaging in advocacy to ensure that vitamin A health is given appropriate consideration within programmes to improve health, and social and economic development.

130. The ecological and related indicators could be collected in the course of any demographic, socioeconomic or health and nutrition surveillance activity. In practice much of the information is probably already available through existing national surveillance systems.

131. Some relevant ecological and related indicators, which can be measured in surveys or surveillance programmes, include:

- ◆ nutritional status information, food availability, food-consumption patterns and levels of intake among vulnerable groups;
- ◆ illness-related indicators;
- ◆ socioeconomic indicators.

132. While all these indicators may not be measured in any single survey, a composite of several from each of the three categories could be measured, or obtained from existing data, and evaluated.

133. Relatively little research has been done to evaluate the relationship between ecological and related indirect indicators and VAD. The proposed interpretations given to them in this section, therefore, should be viewed as suggestive. They will need modification both in the light of further field testing, and in consideration of local contexts.

4.2 Nutritional status and diet-related indicators

4.2.1 Breast-feeding patterns

134. A pattern of breast-feeding to 18 months, inclusive of vitamin A-containing complementary foods from 6 months onwards, is protective against clinical VAD even beyond the time of cessation of all breast-milk feeds.⁶³ Vitamin A-containing complementary foods, or vitamin A supplements, are needed from about 6 months of age to maintain adequate body stores, particularly where mothers themselves are malnourished.⁶⁴

135. Feasibility and performance. Data on breast-feeding patterns are often available in demographic and health surveys (DHS), or other surveys, e.g. related to implementation of the joint WHO/UNICEF Baby-friendly Hospital Initiative (BFHI).

136. Suggested interpretation. Where maternal malnutrition is prevalent, there is a risk of VAD in infants when: (a) $\geq 50\%$ are not receiving breast milk at 6 months of age; (b) when $< 75\%$ of 6-17 month olds do not receive vitamin A-containing complementary foods at least three times per week.

4.2.2 Anthropometric indicators of PEM

137. Stunting (< -2 Z-scores for ht/age) is a surrogate measure of chronic dietary deprivation and other environmental factors that are associated with VAD. Wasting (< -2 Z-scores for wt/ht) is a measure of inadequate food intake, which is also associated with increased risk of VAD.

⁶³ Mahalanabis D. Breast feeding and vitamin A deficiency among children attending diarrhoea treatment centre in Bangladesh: a case-control study. *British Medical Journal*, 1991, 303:493-6.

⁶⁴ (a) Brown KH, Akhtar NA, Robertson AD, Ahmed MG. Lactational capacity of marginally nourished mothers: relationships between maternal nutritional status and quantity and proximate composition of milk. *Pediatrics*, 1986, 78:909-19;

(b) Mele L, et al. Nutritional and household risk factors for xerophthalmia in Aceh, Indonesia: a case/control study. *American Journal of Nutrition*, 1991, 53:1460-5.

138. Feasibility and performance. Anthropometric data are available from nearly all countries. WHO has recently established updated age and sex-specific reference criteria for interpreting anthropometric indices.⁶⁵

139. Suggested interpretation. A prevalence of stunting (ht/age) $\geq 30\%$ in children under 5 years, and wasting (wt/ht) $\geq 10\%$ in children under 5 years identifies a high-risk area/population for vitamin A deficiency.⁶⁵

4.2.3 Prevalence of low birth weight (LBW)

140. A high prevalence of LBW (<2.5 kg) reflects maternal undernutrition. It also suggests the likelihood that body stores of vitamin A, limited in all newborns, are even lower or virtually absent.⁶⁶ LBW infants are thus at increased risk of VAD not only at birth but subsequently if they are breast-fed by malnourished mothers in areas of endemic VAD.

141. Feasibility and performance. Current data may be difficult to obtain from existing local demographic information. WHO has tabulated available global data.⁶⁷

142. Suggested interpretation. A prevalence of $\geq 15\%$ LBW suggests a high-risk area/population.

4.2.4 Market and household food availability

143. Home and/or market surveys of the availability of vitamin A-containing foods are useful.⁶⁸ At the market level a list of the important vitamin A-containing foods purchased by communities, and a rough estimate of quantities available by season and their unit costs for the size of the population served by the market can be collected from interviewing vendors. The focus should be on green leafy and yellow vegetables, yellow fruits, red palm oil where available, and vitamin A-rich animal products (e.g. animal liver, whole milk and eggs).

144. Surveys at the household level should first use focus groups of women and elders to generate a list of important foods available in homes whether from the market, home production, hunting and gathering, or sources outside the community. Information collected should include seasonal availability, usual quantity acquired and prepared for the family, and frequency of use. A random

⁶⁵ de Onis M. et al. The worldwide magnitude of protein-energy malnutrition: an overview from the WHO Global Database on Child Growth. *WHO Bulletin*, 1993, 71:703-12.

⁶⁶ (a) Wallingford JC, Underwood BA. Vitamin A deficiency in pregnancy, lactation, and the nursing child. In: Baurenfeind JC (ed), *Vitamin A Deficiency and its Control*. Academic Press Inc., pp 101-52, 1986.;

(b) Shenai J, et al. Plasma vitamin A and retinol binding protein in premature and term neonates. *Journal of Pediatrics*, 1981, 99:302-5.

⁶⁷ Low birth weight. *A tabulation of available information*. World Health Organization, Geneva, 1992.

⁶⁸ Kuhnlein HV, Pelto GH (eds). Community assessment of natural food sources of vitamin A: Guidelines for ethnographic studies (in press), International Nutrition Foundation for Developing Countries (INFDC), Boston, MA, 1996.

selection of households should be visited at different seasons to see if any vitamin A sources, e.g. dark-green leafy vegetables and orange-coloured vegetables and fruits, are available and actually eaten by young children.⁶⁹

145. Feasibility and performance. Surveys of market and household food availability require the participation of a local assistant guided by a nutritionist. Adequate training of staff as good facilitators and recorders at focus group discussions is crucial. Facilitators should speak the local language. Urban areas call for consideration to be given to the specific shopping districts utilized by the sampled communities.

146. Suggested interpretation. A community is at high risk when vitamin A and carotene-rich foods become scarce in the market and cost to meet the recommended dietary intake by eating them nears the cost of obtaining vitamin A from animal sources. A low risk exists when vitamin A-rich foods are available in the home ≥ 3 -times per week for $\geq 75\%$ of households. In addition adequate amounts of staples and fat/oil should be consumed. The risk of VAD is greater when consumption of foods of animal origin does not regularly occur.

4.2.5 Dietary patterns of vulnerable groups

147. General focus group discussions with women and elders in communities are useful to obtain information on this risk indicator. Emphasis should be on regular food-use practices during pregnancy and lactation, complementary feeding of infants, and post-weaning diets of the young child. Information is needed on food preparation, frequency of consumption in season, and usual portion sizes, and should include an estimate of what proportion of the population follows each practice. This information is most useful when obtained from a community or region that has been stratified by ethnic group, ecological zone, socioeconomic status, and specific vulnerable group.

148. Feasibility and performance. Obtaining information requires the cooperation and good will of local leaders and skilled focus group facilitators with nutrition experience who are familiar with the local language and customs. The reliability of the information is highly dependent on local cooperation and the skill of the focus group leader, including knowledge of the provitamin A-containing foods in the area.

149. Suggested interpretation. An adequate dietary pattern among vulnerable groups means consumption of vitamin A-rich foods three or more times per week together with the use of sufficient staples, fats and some foods of animal origin in $\geq 75\%$ of the population. At-risk groups include pregnant and lactating women, infants and children during the period of complementary feeding, and fully weaned children through 5 years of age.

⁶⁹ Tarwotjo I, et al. Dietary practices and xerophthalmia among Indonesian children. *American Journal of Clinical Nutrition*, 1982, 35:574-81.

4.2.6 Semi-quantitative and qualitative measures of food consumption

150. There are methodologies for preparing rapid assessments (qualitative and semi-quantitative) of food consumption in relation to vitamin A-containing foods, both on a household basis and for specific at-risk groups.⁷⁰ The rapid survey procedures obtain a history of the usual frequency of vitamin A-containing foods consumed in households or by individuals over a defined period, e.g. a day, week or month. This is not a survey of actual quantity of individual foods consumed, but with trained interviewers, semi-quantitative estimates can be made. The primary purpose is to determine whether vitamin A-containing foods are consumed frequently enough in families as a whole, or by at-risk individuals within families, to meet their probable needs, i.e. to rank them as being at risk of inadequate intake. Consumption data provide supportive information for biological indicators for identifying high-risk populations, for forming process indicators of food-based interventions and for tailoring food-based intervention programmes to the context in which they will be implemented.

151. Limitations. A recall method gives quick and useful information, but:

- ◆ it represents consumption patterns over only a limited time (usually a week or less), it can be atypical of usual diet (especially in case of illness or on festive occasions), and it can miss seasonal variations;
- ◆ the data provide only for consumption frequency, unless surveyors are trained to approach consumption levels semi-quantitatively;
- ◆ random sampling is essential to avoid bias in villages selected and households interviewed;
- ◆ if there is a special market in the village on a particular day, the timing of the visit in relation to the market may influence the results;
- ◆ it does not directly provide information on why certain foods are or are not given (e.g. before a certain age).

152. The mother (or other care-giver) of each child of the age range chosen in the randomly selected households should be interviewed in the home or in a neutral public place, and not in a health facility or by a regular health worker since this would likely produce biased answers. The interviewer should be fluent in the language of the person being interviewed and questions should be asked in the vernacular. Care must be taken to avoid asking leading questions or prompting replies.

153. Feasibility and performance. The information should be obtained from populations that have been sufficiently sampled by ethnic group, socioeconomic status and ecological zone to ensure representation of all at-risk groups. Local interviewers, trained and monitored by a nutritionist, are crucial. Performance as an indirect indicator of VAD is dependent on the skills of interviewers and the availability of reasonably complete regional food-composition data. It is not necessary to know every detail, however, e.g. the carotenoid content of individual dark-green leafy vegetables.

⁷⁰ (a) Underwood BA, et al. *Guidelines for the development of a simplified dietary assessment to identify groups at risk for inadequate intake of vitamin A*. IVACG/ILSI Nutrition Foundations, Washington D.C., 1989;

(b) Rosen, DS *Conducting a qualitative assessment of vitamin A deficiency. A field guide for program managers*. Helen Keller International Vitamin A Technical Assistance Program, New York, 1992.

154. Suggested interpretation. The approach measures in a qualitative or semi-quantitative manner actual frequency of vitamin A-containing food intake by vulnerable groups (as opposed to food availability and usual behaviour). The data are useful for population assessment but not for identifying the needs of the individual. The data are analysed by food groups according to the age of the child. This can be quickly done manually using the master table appropriately adapted to the particular survey shown in Annex 2. From this table the frequency of consumption of each category of food, and by each age and physiological group, can be calculated. When foods with high vitamin A content are consumed <3 times weekly in $\geq 75\%$ of the vulnerable groups, there is a high risk of inadequate vitamin A status.

4.2.7 Beliefs and attitudes concerning food

155. Information about beliefs and attitudes related to food is best obtained by using ethnographic techniques such as focus-group discussions with women and elders in the communities surveyed, key informant interviews or observations.^{68,71} Focus groups should be stratified by ethnic group, socioeconomic status and ecological zone, and should include separate sessions for each vulnerable group (i.e. pregnant and lactating women, and mothers of infants and preschool children), depending on the quantity of information and discussion time needed. Focus-group discussion should include beliefs about and attitudes towards vitamin A-rich foods and their relationship to health in general, as well as for eye health specifically. It should seek ideas from the community about how best to improve availability and use of vitamin A-rich foods and foods containing auxiliary nutrients. This approach is dependent on interest and support from local leaders and the skill of focus-group facilitators.

156. Suggested interpretation. This information will greatly assist in developing dietary intervention programmes.⁷¹ It is essential for developing appropriate materials for education and social marketing programmes to change food consumption patterns. Quantitative evaluation of this type of information is difficult and should only be done within specific ethnic, sex or age groups who hold common beliefs.

4.3 Illness-related indicators

157. A general picture of the prevalence among the preschool-aged population of the main relevant illnesses (PEM, measles, diarrhoeal and respiratory diseases); immunization status, especially against measles; and anthropometric status is usually available from demographic survey reports that are often obtained from government offices. In their absence, some information can be obtained by adding simple questions to a dietary questionnaire, for example as shown in Annex 3. However, it may be difficult to establish a child's immunization status outside a health facility (e.g. if a child has had measles vaccination) unless the mother holds the child's growth chart or immunization card on which such information is recorded.

⁷¹ Culture, environment and food to prevent vitamin A deficiency. Kuhnlein, HV and Pelto, GH (eds), (in press), International Nutrition Foundation for Developing Countries (INFDC), Boston, MA, 1996.

4.3.1 Immunization coverage rates

158. Surveillance and reporting using a standard cluster sampling design is routine in most EPI programmes worldwide. This information is available in most countries and need not be collected in special VAD surveys. Coverage rates are an indirect indicator of health system management, especially when the EPI programme is integrated into primary health care.

159. Suggested interpretation. The risk of VAD is increased when coverage rates for full immunization, particularly for measles, fall below 50% for 12-23 month-old-children.

4.3.2 Measles case fatality rate (CFR)

160. The association established in institutional settings between measles severity and VAD makes the measles CFR a possible marker for populations in which VAD poses a serious public health problem. Although clinical studies have clearly delineated the relationship between measles CFR and VAD, more epidemiological studies are needed to measure the exact sensitivity of poor vitamin A status in the community and high measles CFR.

161. Feasibility and performance. The routine collection of the community measles CFR can be problematic unless community health workers have been specifically trained to recognize measles symptoms and collect relevant information.⁷² Information on the measles CFR in an area can be obtained from clinic and district hospital records but this may provide biased results. Data gathered this way, from existing records for which the process of collection is already in place, is therefore a cost-effective approach when the objective is to identify high-risk population of VAD, and measles-related blindness and mortality. Although more serious cases tend to go to hospital, they also receive better treatment.

162. Suggested interpretation. A measles CFR of $\geq 1\%$ in community, clinic or district hospital records denotes a high-risk area. Given the clear relationship between vitamin A status and measles CFR, it is recommended that vitamin A intervention be undertaken in any community/population where the measles CFR exceeds 1%.

4.3.3 Disease prevalence rates

163. Disease prevalence rates, particularly diarrhoeal disease and febrile infections, are associated with depressed appetite, depressed absorption efficiency, and/or increased metabolic utilization and urinary loss of vitamin A.⁷³ Their prevalence can assist in identifying high-risk areas/populations.

⁷² Generic protocol for determining measles case fatality rates in a community either during an epidemic or in a highly endemic area. Expanded Programme on Immunization, World Health Organization, WHO/EPI/GEN/93.3, Geneva, 1993.

⁷³(a) Alvarez JO, et al. Urinary excretion of retinol in children with acute diarrhea, *American Journal of Clinical Nutrition*, 1995;61:1273-6;

(b) Stephensen CB, et al. Vitamin A excreted in the urine during acute infection. *American Journal of Clinical*

Diarrhoea episode rates (incidence/prevalence)

I 64. The synergistic relationship between diarrhoeal disease (DD) and VAD has been the focus of many epidemiologic morbidity and mortality studies where vitamin A is concerned. The impact of vitamin A supplementation on diarrhoea incidence rates continues to be investigated. An increased risk of VAD, the severity of the diarrhoeal episode, and hence the risk of fatality has been associated with DD at individual and population levels.⁷⁴

I 65. While assessment of DD incidence in a region may be helpful in identifying populations at high risk of VAD, its prime usefulness lies in applying such information for disease targeting of vitamin A control programmes, and for monitoring and evaluating control strategies that promote breast-feeding and healthy complementary feeding practices. The available data suggest that providing vitamin A supplements may not influence the severity of a diarrhoeal episode. However, contact with the medical system for a current episode permits administration of a vitamin A supplement that will improve the subject's vitamin A status and decrease the severity of the next episode.

I 66. Feasibility and performance. Data collected by interview on the incidence and period-prevalence of DD, although obtained in most cross-sectional health surveys, are not always reliable given the inverse relationship between accuracy and period of recall. The terminology defining diarrhoeal episodes varies considerably between groups and regions. The case definition of DD requires initial qualitative analysis of local terms and perceptions related to DD for standardization prior to beginning community-based interviews.

I 67. Suggested interpretation. Quantitative information on DD episodes, duration, frequency of stooling, and consistency of stools can be used to determine prevalence of persistent and severe diarrhoea. A two-week period prevalence of DD $\geq 20\%$ in the selected population may be used to identify populations at risk of VAD. However, this criterion should be further validated by field studies that more directly measure vitamin A status among populations with different period-prevalence rates.

Fever and helminthic infection rates

I 68. Both fever—irrespective of etiology—and heavy worm loads usually depress appetite and nutrient absorption efficiency and retention. Both conditions are also associated with depressed circulating blood levels of vitamin A. In febrile conditions, there is evidence of an increased urinary loss of vitamin A which suggests that frequent febrile states contribute to VAD.⁷³ When period-prevalence rates are high, therefore, risk of VAD is expected to increase.

Nutrition, 1994;60:388-92.

⁷⁴(a) Ghana VAST Study Team. Vitamin A supplementation in northern Ghana: effects on clinic attendances, hospital admissions, and child mortality. *Lancet*, 1993;342:7-12;

(b) Barreto, ML et al. Effect of vitamin A supplementation on diarrhoea and acute lower-respiratory-tract infections in young children in Brazil. *Lancet*, 1994;344:228-231.

169. Suggested interpretation. In preschool-aged populations, a two-week fever period prevalence of $\geq 20\%$ indicates an area/population at increased risk of VAD, as does prevalence of helminthic infection of $\geq 50\%$ in the preschool-aged group.

4.4 Socioeconomic indicators

170. Socioeconomic indicators are useful as pointers to a population's vulnerability to VAD and, as such, are useful in planning a vitamin A intervention programme, particularly when no clinical, biochemical or dietary surveys have been done. However, and despite their possible use for advocacy purposes, because these indicators lack both sensitivity and specificity they are of no use in monitoring and evaluating the impact of a vitamin A programme. Though the consultation had no solid basis for establishing relevant interpretation criteria in determining risk of VAD, it made some suggestions that could assist in the relative ranking of areas/populations. These indicators are most useful as supporting evidence of other, more specific, biologic and ecologic indicators of vulnerability to VAD.

4.4.1 Maternal education and literacy

171. There is a consistent inverted relationship between VAD prevalence and maternal education and literacy. Possible indicators include:

- ◆ % of literacy among adult women;
- ◆ % of school attendance by girls aged 6-12 years;
- ◆ adequacy of school curriculum on the causes, manifestations, diagnosis, management and prevention of VAD.

172. Such data are usually available nationally, but not necessarily disaggregated to provincial or district levels. They are nevertheless useful as a general guide to areas where one may expect to find VAD, and to improving the prospect of success in promoting VAD control.

173. Suggested interpretation. No schooling or illiteracy in $>50\%$ of women 15-44 years of age is associated with a high vulnerability to VAD when evaluated along with food availability and dietary patterns of preschool-aged children.

4.4.2 Income/employment

174. In many countries VAD prevalence is closely correlated with poverty. Low-income families are unable to afford frequent consumption of foods of animal origin, the vegetables and fruits that are the main non-animal sources of dietary vitamin A or fat, which facilitates absorption of provitamin A sources. Low income, coupled with the usual lack of knowledge about VAD and the nutrient content of common foods, contribute to a very high risk of VAD. Since reliable income data, are difficult and costly to obtain and analyse, alternative data which may be easier to collect, essentially from urban populations, include:

- ◆ % of households having regularly employed member(s)
- ◆ % of household income spent on food.

175. Collecting such information in a simple VAD survey may be unwarranted, but it could already be available from, or included in, national household surveys, food-consumption and budget surveys, general nutrition or specific micronutrient surveys, or the framework of a health or socioeconomic survey or surveillance system.

176. Suggested interpretation for high risk might be:

Households: no regular employment (urban family) with >70% of income spent on food
 Community: >50% households spend >70% of income on food

4.4.3. Water supply and sanitation

177. The main indicators would be the classic ones for water supply and sanitation:

- ◆ % of households (i) urban (ii) rural having an adequate and safe water supply
- ◆ % of households (i) urban (ii) rural having adequate sanitation (human-waste disposal)

178. These data are commonly collected by national health authorities on an annual or periodic basis, often with the support of external agencies including international and bilateral organizations. A quite comprehensive system of reporting was instituted in the framework of the International Water and Sanitation Decade (1981-1990), often disaggregated to the provincial or even district level. For the most part these data are already available from other reporting systems within countries.

179. Countries have usually adopted their own criteria by which water supply and sanitation coverage is considered "low" or "adequate". Low percentages for these indicators point to low-income populations at high risk of infections from water- and faecal-borne diseases, especially diarrhoeal diseases or ascariasis, which are liable to provoke frank vitamin A deficiency. This situation requires concurrent measures to improve environmental health—including food safety and personal hygiene—in any action programme to improve vitamin A status.

180. Suggested interpretation: There is a high probability of VAD if, in addition to being at risk based on diet and illness-related factors, <50% of households have a safe water supply.

4.4.4 Access to health and social services

181. Populations physically remote from health and social services are often more prone to VAD, partly because of low socioeconomic status, but also because of frequent or severe infections due to low immunization rates and inadequate treatment. Some low-income urban/periurban households, which may make little use of health services, even when available, are likewise at risk.

182. Feasibility and performance. Health services generally have data available, on a provincial or district basis, for the percentage of the population within a given distance from a health unit. These data would suffice to define relatively poorly served areas and populations, and it would thus not be necessary to make collection of this information a special part of vitamin A programmes.

183. The proposed indicators relate to:

- ◆ distance of the population from the nearest health unit;
- ◆ infection-related service indicators like immunization rates (these are covered in the preceding section) and % use of ORS;
- ◆ utilization of MCH services/1000 population in peripheral health units;
- ◆ availability of nutrition education about VAD.

184. Only data on nutrition education activities could be anticipated to have some direct relation to VAD prevalence. VAD could still occur in the presence of such activities, i.e. reports on activities could be positive even though the relevance and efficacy of the activities may be low.

185. Suggested interpretation. These data primarily indicate only populations generally at high risk, particularly if the services mentioned are completely unavailable. Populations living in poverty > 10 km from services are hardly likely to benefit significantly from them unless there is some form of special outreach. The food system should be evaluated to confirm a population's vulnerability.

4.4.5 Access to land

186. In rural areas absence of land ownership, or of access to land, commonly means casual-labour livelihood and, as a consequence, poverty. Traditional practices may allow tenant farming to continue on unfavourable terms for the tenant but without the possibility of producing enough food to meet the family's direct consumption needs. Criteria for "inadequate access to land" for ultimate family food availability have to be defined in each context.

4.4.6 Access to agricultural services and inputs

187. Access to agricultural services and inputs may be of some use as a VAD risk criterion. However, in most countries, where there is no specific agricultural policy in relation to vitamin A, families without access to these services will be mainly at risk only because of possible greater poverty. In the case of a vitamin A programme based on dietary improvement, agricultural extension services could and should play a major role. Process or operational indicators relating to promoting vitamin A-rich foods need to be defined for example:

- ◆ national policy on production and accessibility of vitamin A-containing foods;
- ◆ agricultural extension (educational) activities on vitamin A-containing foods;
- ◆ agricultural extension inputs relating to production of vitamin-A containing foods, e.g. seeds, fertilizers, cuttings, credit, etc.
- ◆ access to these services, e.g. in village itself, or within 10 km;

- ◆ promotion of vitamin A-containing foods and related nutrition education in school and/or community garden programmes;
- ◆ number of varieties of DGLV and other carotene-containing crops existing per home garden/fields, per household in the community;
- ◆ proportion of households in pastoral areas having their own milk-producing livestock.

I 88. Once policies have been adopted on the above-mentioned approaches and activities and goals defined, it should be relatively straightforward to establish detailed operational monitoring and evaluation systems. The relevant indicators are expected to have low sensitivity and specificity before a policy and programme have been adopted. Once adopted, however, they become highly pertinent indicators for monitoring and evaluation purposes.

I 89. Ecological indicators useful for identifying and ranking areas or populations at risk of VAD are summarized in Table I 5. The potential uses of ecological indicators and ranking of their usefulness for different VAD surveillance programmes are also summarized in the table.

Table 15
Ecological indicators and their potential uses in VAD surveillance

	Identification of high-risk groups/areas	Planning VA control programme	Monitoring/evaluating interventions				Advocacy for VA Interventions
			DI ¹	FF ²	SU ³	PH ⁴	
Food availability/dietary/nutrition indicators							
VA food availability including seasonality	+++	+++	+++	+			+++
Food security	+++	+++	+++				
Dietary patterns stratified by ethnic/socioeconomic group, and ecological zones	+++	+++	+++	+			++
Food consumption	+++	+++	+++	+++			+++
Beliefs/attitudes about food		++++	+++	+			+++
Growth indicators (Ht/age; Wt/Ht)	+		+			+	
Illness-related indicators							
Immunization coverage	+					+	
Measles case fatality rate	+	++				+	+
Diarrhoea episodes	+	++	+++			+	+
Fever	+	++				+	
Helminthic infection rate	+	++				+	
SES indicators							
Income/employment	++	+					
Water supply and sanitation	+	+					
Health service accessibility	+	++			++	++	
Land accessibility/ land ownership	+	+++	++				
Education/literacy	+	+++					
Access to agricultural services	+	++	+				
Refugees	+++						+++
Areas in conflict	++						+++

¹ DI = dietary intake; ² FF = Food fortification; ³ SU = supplementation; ⁴ PH = Public health measures. ⁵ The number of "+" indicates the relative strength of usefulness for the purpose and type of intervention programme indicated.

PART V. INTEGRATED APPROACHES TO CONTROLLING VAD

In order to develop cost-effective approaches in developing a VAD control programme it is helpful to examine possible linkages with other community health or development programmes, e.g. breast-feeding, immunization, iodine and iron deficiency control, primary health care, family planning, literacy, agricultural extension and community development, and a variety of programmes involving women, their organizations, local-level political parties, etc. In addition, schools can be an invaluable focal point for promoting vitamin A-containing foods through school gardens, and formal and non-formal educational curricula. An important consideration is to ensure that primary and secondary school teachers, and other community leaders, have sufficient teaching knowledge about vitamin A, the health consequences of VAD and practical local solutions. Awareness and knowledge are crucial to creating a local demand and sustained political commitment by instituting appropriate control measures.

190. VAD control activities fall into three major categories: improved dietary diversity and quantity, improved dietary quality through food fortification, and appropriate amount and frequency of supplements. It is usually appropriate to promote a mix of these activities coupled with public health measures to reduce the prevalence of infections and economic programmes to improve income and, hence, access to food, all tailored to local circumstances.⁷⁵

5.1 Improving dietary quality and quantity through dietary diversification

191. The goal is to achieve continually adequate intake, defined as the recommended dietary intake (i.e. RDA or RDI) of vitamin A-containing foods in the context of an adequate total diet. A strategy to carry out this goal may draw upon a combination of horticultural, agricultural and educational inputs, food fortification, socioeconomic and social marketing (IEC) approaches, which in practice means that an intersectoral approach is crucial. Under most circumstances, sustained breast-feeding, while ensuring adequate vitamin A intake by mothers, remains the best protection against severe VAD throughout the complementary feeding years. Consideration should be given to developing IEC materials that concurrently promote foods rich in vitamin A that also contain iron and iron absorption enhancers (vitamin C), since iron deficiency is so widespread among the same VAD vulnerable populations. Given the synergistic relationship between VAD and infectious and parasitic diseases, and interactions with other nutrients, it is also advisable to address simultaneously the control of diarrhoeal disease, helminthic infections, measles and PEM, and to improve levels of community sanitation.

⁷⁵ Subcommittee on Nutrition. *Controlling vitamin A deficiency*. A report based on the ACC/SCN Consultative Group Meeting on Strategies for the Control of Vitamin A Deficiency, 28-30 July 1993, Ottawa, Canada. ACC/SCN State-of-the-Art Series. Nutrition Policy discussion Paper No. 14, January 1994.

5.2 Improving dietary quality through food fortification

192. Food fortification means the addition of vitamin A to an appropriate dietary vehicle. Fortification of condiments and foods such as MSG, sugar and margarine is another major intervention strategy which, after start-up costs have been met, can be relatively inexpensive, sustainable and effective. Efforts are under way to identify food vehicles that may be fortified with multiple micronutrients, particularly iron and iodine. The advantage of this strategy is that active consumer participation is minimal, since the consumer continues to eat a food that is now fortified. One of the first steps is to identify a technically fortifiable food vehicle that is consumed in adequate amounts within a relatively narrow range by the at-risk population, manufactured or processed in only a few locations, and retains its potency and acceptability after being fortified. Probing for potentially fortifiable food vehicles should form part of focus-group discussions to determine food availability, dietary patterns and food beliefs and attitudes. Although food fortification appears to be relatively straightforward, it has proven difficult to implement and sustain in countries where a food processing industry is not developed, which is frequently where the most vulnerable populations are located. The greatest success has been achieved in countries that have a middle level of development and a high proportion of urban population.

193. Another approach that has not been adequately pursued is food-to-food fortification, which involves combining a vitamin A-rich food in concentrated form with a staple food that has low vitamin A content. An example is solar drying of mangoes or green leafy vegetables in season, preserving them (perhaps in crushed or pulverized form), and combining the rehydrated product with cereals or paps used to feed infants and young children during the complementary and post-weaning feeding periods.

5.3 Supplementation

194. Supplementation is the delivery of vitamin A, commonly a high dose given orally, on a periodic basis, usually every 4–6 months. This control strategy is the most immediate and direct approach to improving vitamin A status and the one most widely implemented. Although oral supplementation with vitamin A can be used for both treatment and prevention of VAD, it is not recommended as a long-term solution unless doses at the level approximating the RDI can be integrated into a community-based delivery system. The objective of this approach is to achieve wide coverage of the selected group in areas of endemic VAD throughout the high-risk years by a system that is culturally acceptable, administratively feasible, and economically practical. Where it is feasible to provide supplements at near-physiological levels at frequent intervals —perhaps weekly at 5000–10 000 IU, or monthly at 25 000 IU— this approach should be considered. Low-dose supplements have greater potential in community control programmes, and hence are adaptable to a variety of existing infrastructures where coverage and outreach may be greater than that available through the formal health system.

5.4 Public health measures

195. Although public health measures alone are unlikely to eliminate VAD, they can render more effective other intervention programmes, particularly food-based approaches, that seek to improve vitamin A intake. Thus, measures involving primary health care personnel, including those providing sick-child and immunization services, are a critical part of the prevention team and should be fully included in all aspects of community VAD control. Decreasing disease prevalence through immunization, particularly measles, parasite control (e.g. deworming) and other control and sanitation programmes will contribute both directly and indirectly toward VAD prevention as channels for the delivery of vitamin A supplements and nutrition guidance, as appropriate.

196. Process indicators to monitor programme implementation in one or all of these areas have to be developed in each country, in accordance with the specific approaches and targets adopted in the national or local plan of action.⁷⁶ Some generic examples of process indicators are given in Table 5. The impact of any of the programmes, however, can be evaluated only by using biological indicators.

⁷⁶A manual for monitoring vitamin A deficiency elimination programmes is being developed by the Programme Against Micronutrient Malnutrition (PAMM), 1518 Clifton Road, NE, Atlanta GA 30322, USA and the Micronutrient Initiative (MI), BP 8500 250 rue Albert, Ottawa, Canada K1G 3H9.

ANNEX I. COUNTRIES CATEGORIZED BY DEGREE OF PUBLIC HEALTH IMPORTANCE OF VITAMIN A DEFICIENCY, BY WHO REGION

(From information available to WHO as of 1996)

WHO region	Clinical	Subclinical			No data available	VAD under control/ no problem likely
		Severe	Moderate	Mild		
Africa	Angola Benin Burkina Faso Cameroon Chad Comoros Ethiopia Ghana Kenya Malawi Mali Mauritania Mozambique Niger Nigeria Rwanda South Africa Swaziland Togo Uganda United Rep. of Tanzania Zambia Zimbabwe	Burundi Cape Verde Congo Côte d'Ivoire Gambia Lesotho Senegal	Botswana Eritrea Namibia Sierra Leone	Madagascar	Algeria Cent. African Republic Dem. Rep. of the Congo Equatorial Guinea Gabon Guinea Guinea-Bissau Liberia Mauritius Sao Tome & Principe Seychelles	
Americas	Dominican Republic Haiti	Brazil Colombia El Salvador Mexico Nicaragua Peru	Belize Bolivia Ecuador Guatemala Honduras	Guyana Panama	Argentina Cuba Dominica Paraguay Puerto Rico* Suriname Uruguay Venezuela	Antigua & Barbuda Bahamas Barbados Canada Chile Costa Rica Grenada Jamaica St. Kitts & Nevis St. Lucia St. Vincent & the Grenadines Trinidad & Tobago United States of America
South-East Asia	Bangladesh Bhutan India Nepal Sri Lanka	Indonesia Myanmar	Thailand		Maldives	Democratic People's Rep. of Korea

*Associate Member

(Cont.)

WHO region	Clinical	Subclinical			No data available	VAD under control/ no problem likely
		Severe	Moderate	Mild		
Europe				Israel Romania Turkey Uzbekistan	Albania Armenia Azerbaijan Belarus Bosnia & Herzegovina Bulgaria Croatia Czech Republic Estonia Georgia Hungary Kazakstan Kyrgyzstan Latvia Lithuania Malta Republic of Moldova San Marino Slovakia Slovenia Tajikistan The former Yugoslav Rep. of Macedonia Turkmenistan Ukraine Yugoslavia	Austria Belgium Denmark Finland France Germany Greece Iceland Ireland Italy Luxembourg Monaco Netherlands Norway Poland Portugal Russian Fed. Spain Sweden Switzerland U.K. of Great Britain & Northern Ireland
Eastern Mediterranean	Iraq Somalia Sudan Yemen	Afghanistan Pakistan	Djibouti Egypt Islamic Rep. of Iran Oman	Jordan Lebanon Libyan Arab Jama. Saudi Arabia Syr. Arab Rep. Tunisia Un. Arab Emirates	Kuwait Morocco Qatar	Bahrain Cyprus
Western Pacific	Cambodia Kiribati Marshall Isl. Micronesia (Fed. States of) Papua New Guinea Philippines Solomon Isl. Vanuatu Viet Nam	Lao P.D.R.	China Malaysia		Cook Islands Mongolia Nauru New Zealand Niue Palau Tonga Tokelau* Tuvalu	Australia Brunei Darussalam Fiji Japan Rep. of Korea Samoa Singapore

* Associate member

Annex 2 Sample form for weekly frequency of complementary feeding by food categories

	Complementary feeding by age group ¹					Family ²	
	0-5 mo	6-11 mo	12-23 mo	24-35 mo	36-71 mo	Yes	No
Staple							
Foods of animal origin							
DGLV							
Yellow fruit/vegetable							
Oil/fat							
Other							

¹ Enter the number of times per week each category is consumed. Data to be compiled by food consumption recalls or food frequency questionnaires.

² Similar format may be used for other vulnerable groups, e.g. pregnant or lactating women.

Sample evaluation guide form

(To be completed for each of the five age groups and family [or pregnant or lactating woman])

Age group (family)		0-5 mo	6-11 mo	12-23 mo	24-35 mo	36-71 mo	Family
DGLV	< 3 x/wk						
	≥ 3 x/wk						
Yellow fruits/vegetables	< 3 x/wk						
	≥ 3 x/wk						
Foods of animal origin	< 3 x/wk						
	≥ 3 x/wk						
TOTAL	< 3 x/wk						
	≥ 3 x/wk						

Annex 3**Sample of survey form for individual child/family**

(Children 6-71 months)

Name of village:

District:

Name:

Mother's name:

Father's name:

Occupation of mother:

Occupation of father:

Date of birth (child):

Age: (years/months)

Presently breast-feeding: Yes () No ()

If not breast-fed, at what age was breast-feeding stopped: months of age

Foods taken during the last day				
By child ¹				By other family members
	6-17 mo	18-35 mo	36-71 mo	
<u>Morning</u>				
<u>Mid-day</u>				
<u>Evening</u>				
<u>Other times</u>				

¹ Circle appropriate age category

Illness that occurred in last two weeks (except for measles in last 6 weeks):

If occurred, check the bracket.

	<u>Yes</u>	<u>No</u>
Persistent cough:	()	()
Fever:	()	()
Diarrhoea:	()	()
Measles (last 6 weeks):	()	()
Other (specify):	()	()
Oedema:	()	()
Obvious anaemia:	()	()
Other abnormal finding (specify):	()	()

Weight (to nearest 0.1 kg): _____ kg

Length (to nearest 0.5 cm): _____ cm

Immunization status

OPV3:

Yes () No ()

Measles: Yes () No ()

Annex 4 Minimum samples sizes for anticipated prevalence with relative precision of 20% and 50% at the 95% confidence level

Anticipated prevalence	Relative precision of 20%		Relative precision of 50%	
	Prevalence range	Sample size	Prevalence range	Sample size
0.01	0.008 - 0.012	960 305	0.005 - 0.015	153 640
0.05	0.04 - 0.06	191 984	0.025 - 0.075	30 718
0.1	0.08 - 0.12	95 944	0.05 - 0.15	15 352
0.5	0.4 - 0.6	19 112	0.25 - 0.75	3 058
1.0	0.8 - 1.2	9 508	0.5 - 1.5	1 522
2.0	1.6 - 2.4	4 706	1.0 - 3.0	753
5.0	4.0 - 6.0	1 825	2.5 - 7.5	292
10.0	8.0 - 12.0	865	5.0 - 15.0	139
20.0	16.0 - 24.0	385	10.0 - 30.0	62
25.0	20.0 - 30.0	289	12.5 - 37.5	47
30.0	24.0 - 36.0	225	15.0 - 45.0	36
40.0	32.0 - 48.0	145	20.0 - 60.0	24
50.0	40.0 - 60.0	97	25.0 - 75.0	16

Annex 5 List of participants

Technical consultation on indicators for
assessing vitamin A deficiency
(Geneva, 9-11 November 1992)

Dr H. Flores
Department of Nutrition
University Federal Pernambuco
50670-901 Recife, PE, Brazil

Mr J. Gorstein
University of Michigan
Ann Arbor, Michigan, USA

Dr P. Greaves
2 The Plantation
London SE3 0AB, UK

Dr H. Kuhnlein
School of Dietetics and Human Nutrition
McGill University, Macdonald Campus
21, 111 Lakeshore Road
Ste Anne de Bellevue
Quebec H9X 4V1, Canada

Dr D. Miller
Nutritional Biochemistry Branch
Center for Environmental Health
CDC
Atlanta, GA 30333, USA

Dr G. Ndossi
Tanzania Food and Nutrition Centre
P.O. Box 977
Dar es Salaam
United Republic of Tanzania

Ms S. Pak
University of Michigan
Ann Arbor, Michigan, USA

Dr S. Resnikoff
Directeur
Institute d'Ophtalmologie tropicale
de l'Afrique (IOTA)
B.P. 248
Bamako, Mali

Dr D. A. Ross
Senior Lecturer, Maternal and Child Health
London School of Hygiene and Tropical
Medicine
University of London
Keppel Street
London WC1E 7HT, UK

Dr R. Stoltzfus
Department of International Health
School of Hygiene and Public Health
Johns Hopkins University
615 N. Wolfe Street
Baltimore, MD 21205, USA

Dr S. Tanumihardjo
Department of Biochemistry and Biophysics
Iowa State University
3258 Molecular Biology Building
Ames, Iowa 50011, USA

Dr E. Udomkesmalee
Institute of Nutrition
Mahidol University at Salaya
Puttamonthon, Nakhon Chaisri
Nakhon Pathom 73710
Thailand

Dr C. E. West
Department of Human Nutrition
Wageningen Agricultural University
Bomenweg 2, PO.Box 8129
6700 EV Wageningen
Netherlands

WHO Secretariat

Dr G. Clugston, Nutrition Unit
Dr K. Bailey, Nutrition Unit
Dr B. Underwood, Nutrition Unit
Dr G. Peltó, Control of Acute Respiratory
Infections
Dr N. Cohen, Expanded Programme on
Immunization
Dr B. Thylefors, Programme for the
Prevention of Blindness
Dr Pararajasegaram, Programme for the
Prevention of Blindness

SELECTED WHO PUBLICATIONS AND DOCUMENTATION OF RELATED INTEREST

MICRONUTRIENT MALNUTRITION

Global prevalence of vitamin A deficiency. Micronutrient Deficiency Information System, MDIS Working Paper No. 2. Geneva, World Health Organization, 1995.

Sommer, A. *Vitamin A deficiency and its consequences: a field guide to their detection and control.* Third edition. Geneva, World Health Organization, 1995. Sw fr. 17.- (11.90).

Using immunization contacts as the gateway to eliminating vitamin A deficiency. A policy document. Document WHO/EPI/GEN/94.9 Rev. 1, Geneva, World Health Organization, 1995.

Vitamin A supplements: A guide to their use in the treatment and prevention of vitamin A deficiency and xerophthalmia. Second edition, Geneva, World Health Organization, (in press), 1996.

Global prevalence of iodine deficiency disorders. Micronutrient Deficiency Information System, MDIS Working Paper No. 1. Geneva, World Health Organization, 1993.

Iodine and Health: eliminating iodine deficiency disorders safely through salt iodization. A Statement by the World Health Organization. Document WHO/NUT/94.4, Geneva, World Health Organization, 1994.

Indicators for assessing Iodine Deficiency Disorders and their control through salt iodization. Document WHO/NUT/94.6, Geneva, World Health Organization, 1994.

The safe use of iodized oil during pregnancy. *Bulletin of the World Health Organization*, 74(1): 1-3.

Indicators for assessing iron deficiency and strategies for its prevention. (In preparation), 1996.

The prevalence of anaemia in women: a tabulation of available information. Document WHO/MCH/MSM/92.2. Second edition, Geneva, World Health Organization, 1992.

NUTRITIONAL ASSESSMENT MONITORING

de Onis et al. The worldwide magnitude of protein-energy malnutrition: an overview from the WHO Global Database on Child Growth. *Bulletin of the World Health Organization*, 1995. 71(6): 703-712.

WHO Working Group on Infant Growth. An evaluation of infant growth: the use and interpretation of anthropometry in infants. *Bulletin of the World Health Organization*, 1995. 73(2): 165-174.

Vitamin A deficiency (VAD) is the single most important cause of childhood blindness in developing countries. It also contributes significantly, even at subclinical levels, to morbidity and mortality from common childhood infections. VAD is the result of two primary factors: persistent inadequate intake of vitamin A that is frequently exacerbated by other dietary circumstances, and a high frequency of infections. An estimated 2.8 million preschool-age children are at risk of blindness from VAD, and the health and survival of 251 million others are seriously compromised.

Heightened awareness of the role of vitamin A in human health has led to an international effort to eliminate vitamin A deficiency and its consequences as a public health problem by the year 2000. This is among the important end-of-decade micronutrient goals endorsed by the World Summit for Children (1990), the International Conference on Nutrition (1992), and the World Health Assembly (1993).

This document is intended primarily for managers of national programmes for the prevention and control of micronutrient malnutrition, particularly vitamin A deficiency. It provides principles governing the use of biological indicators for vitamin A deficiency (VAD) surveillance, provides the rationale behind each indicator and its limitations and cutoff points for interpretation in terms of public health significance. For the first time, a series of non-biological indicators useful to identify high risk areas are provided and cutoff points for their interpretation suggested. Also included are indicators for monitoring progress toward achieving the goal of elimination of VAD as a significant public health problem by the year 2000.