

MALNUTRITION AND MEASLES  
Ig. G. ELISA in 0-23 Months Children  
Comparison of Seroconversion  
And Vaccine Side Effects,  
Addis Ababa  
by  
AMARE ABEBE, MD

Addis Ababa, Ethiopia  
February, 1988

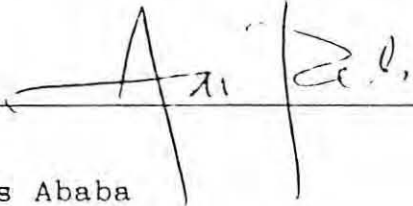
DECLARATION

I, the undersigned, declare that this thesis is my work and that all sources of material used for this thesis have been duly acknowledged.

Name AMARE ABEBE MD.

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Signature



A handwritten signature in black ink, consisting of a large, stylized 'A' followed by 'a', and a vertical line with a flourish to the right.

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Place Addis Ababa

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ADDIS ABABA UNIVERSITY, FACULTY OF MEDICINE

Post Graduate Program in Community Medicine

CERTIFICATE OF APPROVAL

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I, hereby, recommend that the thesis prepared under my supervision by Dr. Amare Abebe Entitled Malnutrition and Measles: Ig G ELISA in 0-23 Months Children Comparison of Seroconversion and Vaccine Side Effects, Addis Ababa be accepted in partial fulfilment of the requirements for the degree of MASTERS OF SCIENCE IN COMMUNITY MEDICINE -M.Sc. (Comm. Med.).

Prof. M.P. Mandara

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MALNUTRITION AND MEASLES  
Ig G ELISA IN 0-23 MONTHS CHILDREN  
COMPARISON OF SEROCONVERSION AND VACCINE SIDE EFFECTS,  
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BY

AMARE ABEBE, M.D.

Thesis submitted as partial Fulfilment of the  
Requirements for the Degree of Master of Science in  
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## ABSTRACT

A cohort of 9 - 23 months old Addis Ababa children were assessed as to their immune response to measles vaccine in relation to their nutritional status. Of the 307 pairs of blood specimens analyzed for measles antibody, 207 (67.4%) had a negative prevaccination titer while 100 (32.6%) had prevaccination titers that were seropositive. Among the seronegatives a 95% seroconversion rate was obtained with no difference between the malnourished and well nourished. There was no difference in side effects reported to the measles vaccine between well nourished and malnourished children. Vaccination of those who were seropositive at the outset didn't results in any booster effect, defined by a four fold rise from the previous titer. Only 9 (9%) of these children showed a booster effect. A validity test for history of measles against the laboratory results gave a 95.1% specificity and 31% sensitivity rate. A second group of children who were under 9 months and were not EPI eligible were also analyzed as to their antibody status . Out of 95 children with analyzable samples 47 (49.5%) had detectable maternal antibody while 48 (50.5%) had no detectable maternal antibodies. The waning sets in at an early age and reaches 100% by 6 -8 months of age.

## I INTRODUCTION

Children are the future of the world and yet they are one of the most vulnerable groups in the population. One of the public health burdens which the developing nations of the world are facing is the alarmingly poor health status of their children. Some of their health indicators are compared below:

Health status indicators *	Developed Countries	Developing Countries
Infant mortality rate (IMR)	15 0/00	200 0/00
Child mortality rate (CMR)	0.4 0/00	100 0/00
Under - 5 mortality rate	2 0/00	>100 0/00
Percentage of low birth weight	4 0/0	up to 50 0/0
Maternal mortality rate (MMR)	0.1 0/00	30 0/00

The leading causes of childhood morbidity and mortality are diarrhea, nutritional deficiency and infectious diseases. Almost 4 million children a year die and another 4 million get permanently disabled from vaccine preventable diseases (1). The World Health Organization (WHO) expanded program on immunization focuses on six diseases; measles, pertussis, neonatal tetanus, tuberculosis and diphtheria.

\* Development of indicators for monitoring progress  
towards Health for All by the year 2000

WHO, Geneva 1981 Number 4

Among these infectious diseases that are the most prevalent and deadly to the child, measles is the most important, alone claiming the life of 2 million children every year, almost 50% of the annual mortality from vaccine preventable diseases. With the development of the measles vaccine in the mid 1960s the disease stopped ranking as a major public health problem in the developed world. The situation was quite different in the developing countries whose children were constantly overwhelmed with a lot of other illnesses which were considered contraindications to measles vaccination. The national EPI policy for Ethiopia mentions no contraindication to measles vaccine on its guideline, though it does not emphasize the need to perform vigorous vaccination activity among the malnourished who experience a high morbidity and mortality from natural measles. In the interim, measles vaccination coverage figures, even from the most well served populations in developing countries, with few exceptions never exceeded the 30% mark (1).

Among the "common contraindications" to measles vaccination is malnutrition which afflicts anywhere up to 40% of the under five population. This meant that up to 40% of eligible children for measles vaccination would have the vaccine withheld because of malnutrition. The percentage will be higher if other illnesses like running nose, fever and diarrhea are considered as contraindications. This phenomenon is observed at the periphery where the actual

vaccination is undertaken. Most hospitals are reluctant to administer vaccines in their wards. This practice goes contrary to current knowledge and necessitates a change in attitude and practice of health personnel with dissemination of knowledge to the periphery.

Several studies have shown that far from being a contraindication to measles vaccination, malnutrition should be considered as a strong indication for measles vaccination. WHO has recommended the same. It is ill or malnourished infants who are most in need of immunization, as they are the ones most likely to die or have serious complications if they contract one of the diseases that could have been prevented (43). This study is undertaken to find out if vaccinating malnourished children has any untoward effects in Addis Ababa and use this information as a basis for reviewing and strengthening its vaccination policy.

## III LITERATURE REVIEW

Measle is an acute exanthematous and enanthematous viral infection. EL - Yehundi, a Hebrew physician, and Rhazes wrote about it in the tenth century, and Sydenham in the seventeenth century wrote a full account of the disease and differentiated it from other exanthems (1,2). Much later measles outbreaks were reported in London and Paris by different authors.

Rubeola is a droplet infection which begins with the inhalation of air-borne particles into the nasopharynx of a susceptible person (3,4). It spreads easily and rapidly requiring a small number of virus particles to establish infection. This makes it one of the most contagious diseases (4). The virus resembles the paramyxovirus and is a single stranded unsegmented RNA virus which was first isolated by Enders and Peebles in 1954 (3,4,5). It has no natural reservoir of infection, but man is the only one from whom the virus was isolated. The disease however has been induced experimentally in the monkey (3,4). The wild virus in nature has no structural varieties (5,9).

Inhaled virus particles penetrate cells of the nasal mucosa diverting their metabolism to the production of new viruses which spread to adjacent cells (6). Infected cells stick together and coalesce into large multinucleated giant cells (6). During the prodromal period around the tenth day, prodromal signs consisting of conjunctivitis, coryza, fever and the familiar koplik spot appear (3,6).

Inflammation of the trachea and bronchial mucosa causes cough. By the 12th day enormous quantities of viruses are released and it is during this catarrhal prodromal stage that the patient is highly infective (4,6). Viremia occurs with a marked rise in fever and misery. The host learns of the presence of measles virus in the first days of infection and two immune reactions begin. These involve both the humoral and cellular immune system reaching their peak of production at about the 14th day (6). The process of virus elimination is usually very efficient with most viruses and giant cells removed within forty-eight hours of the onset of rash (8).

Free of the virus the child quickly recovers and can no longer spread infection, limiting the time of infectivity to about 48 hours before and after the onset of the rash (4,6). The normal course of measles is associated with a balance between viral aggression and host defences. Without a lymphocyte response, whole body infection proceeds quickly to death (8). Infection with natural measles results in a solid lifelong immunity (4,5,6,). In severe forms of the disease, sick children seldom recover in less than 10-14 days after the onset of rash and are prone to secondary bacterial infections and especially infections with unusual bacteria such as the gram negative bacilli (6).

Since there is no tendency towards variation of the measles virus in the wild state, the host response is incriminated as causing the clinical variations observed. Severity of the disease is found in high association with malnutrition and significantly increased fatality rate in this group (10).

There is no specific treatment for measles and gammaglobulin is of no value once symptoms are evident. However, it can be prevented by inducing active immunity through the use of a vaccine.

It was after Enders & his co-workers first demonstrated reliable techniques for the growth and propagation of the measles virus in tissue culture that many scientists managed to come up with a safe and effective vaccine against natural measles. In 1958 the first measles vaccine Edmonston B strain was tried on humans. Further attenuated Schwarz strain vaccine derived from additional chick cell culture passages of the original Edmonston B strain is currently recommended and is associated with few local or systemic reactions (3,5).

A shift in the median age for measles have been noted with the introduction of vaccination programmes. Measles is primarily a disease of childhood and is endemic throughout the world except in isolated populations. Prior to development of an effective vaccine the disease occurred in epidemic cycles every 2 to 3 years. Though it may occur at

any time of the year it is most common in the late winter or early spring corresponding to a low relative humidity. It is well established that alteration of the immune response results in higher susceptibility and ultimately leads to an increased morbidity and mortality. The relation between nutritional deficiencies and immune response is an area of wide interest for researchers. Malnutrition has been reported to cause general morphological changes in the organs of the immune system (11). The primary immune deficiency in malnourished individuals is a decrease in the ability to produce a cell-mediated response (12). In contrast to T-cell activity the B-cell compartment of malnourished individuals remains normal (13). Thus, the pattern of alteration of the immune response resulting from nutritional deficiencies prompt us to evaluate the efficacy of our vaccines. In general one can't predict how a malnourished child responds to vaccines (11).

It has been shown that malnourished children develop normal levels of neutralizing antibody following live polio virus and measles vaccination but secretory Ig A antibody is diminished (11). Several authors have found that malnourished children fail to develop HI antibody following measles vaccination. However, according to other investigators, the development of protective levels of antibody following immunization of children with live measles vaccine was not impaired by malnutrition. Similarly, reports have shown that the antibody response

may be delayed, but by 42 days after immunization with live measles vaccine, 90 percent of malnourished children exhibit protective levels of antibody. Although the immunologic capabilities of malnourished children are impaired, the administration of measles vaccine is effective in these individuals (11).

Different authors have speculated that adverse reactions might occur more often in malnourished children. This is assumed to be due to the depressed cell-mediated immune responsiveness of malnourished children (37). It has been also speculated that the administration of measles or other live viral vaccines to malnourished children might result in latent or slow viral infections such as subacute sclerosing panencephalitis (SSPE). The establishment of a cause and effect relationship in the evaluation of vaccine adverse effects is a complicated one.

There are epidemiological criteria set in an attempt to distinguish causal from non-causal association and temporality, which is a necessity that the cause precede the effect in time, is the only criterion considered a sine qua non of an association (36). Halsey and Stetler<sup>37</sup> claim that reported rates of adverse reactions to vaccine have varied markedly and also list the following factors associated with rates of adverse reactions to vaccines.

## 1. VACCINES

- 1.1. Type of strain
- 1.2. Number of organism or titer of antigen
- 1.3. Media used to grow organism
- 1.4. Inactivation or attenuation process
- 1.5. Adjuvants
- 1.6. Stabilizers or preservatives

## 2. VACCINEES

- 2.1 Age
- 2.2 Sex
- 2.3. Previous dose of vaccine
- 2.4. Prior illness with the offending agent
- 2.5. Passively acquired antibody
- 2.6. Coincidental illness
- 2.7. Immune deficiency

## 3. ADMINISTRATION

- 3.1. Jet gun versus needle and syringe
- 3.2. Injection site
- 3.3. Tissue injected (SC, ID, IM)

In general, live vaccine is believed to have an onset of adverse effects 7-14 days after vaccination while that of the killed (inactivated) vaccine and toxoid tend to occur in 24-72 hrs (41). The rare but serious adverse reactions following measles immunization are encephalitis, encephalopathy, pneumonia, subacute sclerosing panencephalitis, convulsion and death (42).

There was speculation that the administration of measles or other live viral vaccines to malnourished children might result in latent or slow viral infections, subacute sclerosing panencephalitis. However, there is no evidence to support this hypothesis (11). Fever of 39.4°C and fleeting rash are reported in 5-15 percent of recipients of measles vaccine (24).

## IV INTRODUCTION TO THE STUDY

The state of measles in Addis Ababa or in Ethiopia at large is not clear as there is no disease surveillance done. The routine health information is also inadequate. In one paper it was mentioned that the incidence of measles is estimated at 400/1000 (14,15) (Units not specified in original article).

In a retrospective analysis report of admissions to the Ethio-Swedish pediatric hospital (ESPC) in 1963 by Arhamar and Habte<sup>16</sup> and a decade later in 1973 by Taffesse<sup>17</sup>, measles was not in the list of the leading principal diagnoses made.

Taffesse<sup>17</sup> has lumped measles with other infectious diseases and they constitute about 20.2% of the total morbidity. Infectious diseases are placed third on the list contributing to 9.8% of cases of the total admissions.

On the contrary Freij<sup>18</sup> and his associates did a retrospective study of measles cases seen at ESPC in the year 1988 and found out that 4.3% of the admissions were measles or its associated conditions and constitute 6.7% of the total deaths. They also found a 9.3% case fatality rate, and 80% of the deaths were less than 80% of the standard weight on the road to health chart (Harvard). They noticed a peak incidence occurring with an interval of 1 1/2-2 years and no particular seasonality. He finally concluded that 1 (one) out of every 20 patients seen is a measles case both in the wards and in the out patient department.

Considering the seriousness of the disease and taking into account that effective vaccines are available, a nation wide EPI was launched in January 1980 thereby declaring war against the major six vaccine preventable diseases. The objective of the program is to make vaccination services available to all children below 24 months of age and pregnant woman (14). A lot has been done in bringing success to the program in such a short time and yet a tremendous amount of work is remaining to be done if we are to meet the target of 100% coverage by 1990. One area of interest for the program is research development. Among the research priorities mentioned are the study of incidence and prevalence of target diseases, on community participation and cold chain equipment (14,15). In light of all this and the reasons mentioned in the introduction the author has taken the liberty to choose the title malnutrition and measles vaccination in those eligible for the national EPI. As mentioned before there are health workers at the periphery who still consider malnutrition to be an absolute contraindication to measles vaccine.

The study is undertaken with the following objective:

1. To determine and analyze the pattern of antibody development to measles vaccination in children who are malnourished as compared to those wellnourished.

Secondary areas also explored as part of this study were:

1. The rate of occurrence of adverse reaction to measles vaccine in the malnourished versus wellnourished
2. The presence of a booster effect in those who are already seropositive when the measles vaccine is administered
3. The validity of child's history of measles obtained from the mother/guardian as compared to the laboratory results.
4. The waning of maternal antibody

## HYPOTHESES

In this analytical study the following hypotheses are to be tested:

- H<sub>1</sub> : The antibody titer rise in children vaccinated for measles is directly related to their nutritional status.
- H<sub>2</sub> : Frequency of vaccine side effects is inversely related to the nutritional status of vaccinees.
- H<sub>3</sub> : The booster effect is directly related to nutritional status.

## V MATERIAL AND METHODS

## STUDY POPULATION

A cohort of children under the age of two years were included in this study. This gross age grouping had two markedly distinct categories of children relevant to the study. One class was composed of those between 9-23 months old who were eligible for immunization for measles (national EPI program). The second category of children comprised those below 9 months who are not eligible for measles vaccination. All the study population were urban children sequentially analyzed when coming to the study sites (health units) either for immunization, as it is the case for the first group or another type of health service for those who were under 9 months of age. All children who could not present a vaccination card on which was indicated previous measles vaccination were included in the study. Those who need to take a multidose vaccination were excluded. This effectively excluded children with lost cards, as any child of the age eligible for measles vaccine with no card, was assumed to require DPT as well as measles vaccine. Five health units are used as static while another thirteen health units are included by an outreach team much later in the study. We were able to analyze a total of 100 children who are 0-8 months old and 341 children that are 9-23 months (Appendix III).

## SAMPLE SIZE CALCULATION

This was done only for the difference in seroconversion between malnourished and wellnourished ie. difference in proportion in unpaired samples.

Not done for side effects, booster effect or waning of maternal antibody which were secondary areas of interest.

Null hypothesis - both well seroconvert at 95%

$$\text{ie } \bar{\pi}_T = \bar{\pi}_C = .95$$

( $\bar{\pi}_T$  = Malnourished;  $\bar{\pi}_C$  = wellnourished)

$$\bar{\pi} = \bar{\pi}_C$$

Alternate hypothesis - malnourished children convert

at 75%

$$\Delta = \bar{\pi}_T - \bar{\pi}_C = -20\%$$

$$\alpha = 0.05 \quad ; \quad \beta = 0.05$$

$$n = \left[ \frac{Z_\alpha \sqrt{2\bar{\pi}(1-\bar{\pi})} - Z_\beta \sqrt{\bar{\pi}_T(1-\bar{\pi}_T) + \bar{\pi}_C(1-\bar{\pi}_C)}}{\Delta} \right]$$

NB. n is for each  $\hat{\Delta}$  group.

$$n = \left[ \frac{1.96 \sqrt{2(.95)(1-.95)} + 1.96 \sqrt{(.75)(1-.75) + (.95)(1-.95)}}{.2} \right]$$

$$n = 61$$

Because of possible difficulties in interpreting test results, it was decided to try to obtain approximately 100 in each group.

## MEASUREMENTS

Weight was taken using a calibrated salter spring-balance scale after removing excessive garments. The weight for age standard was used for nutritional classification. Those who are above or equal to the 80% of the Harvard standard were taken as well nourished, 60-79% moderately undernourished and less than 60% as severely undernourished (19).

Considering the age of our study population, technical feasibility, and social acceptance, we resorted to the finger prick filter paper disc method for our seroepidemiological study as described by Brody et al<sup>20</sup> and Chin et al<sup>21</sup>. Blood was obtained by the vaccinators after cleaning the pulp of the finger with 70% alcohol, lancing and removing the first drop of blood with a dry cotton wool and soaking the filter paper disc. The disc (whatman filter paper disc grade 3mm, size 1.2 cms made in England) was soaked properly until saturated by making sure that no white spot remained (21). Disposable sterile lancet was used for pricking and disposable glove was worn by the vaccinator when performing the procedure. The discs so obtained are then placed in individual vials (5-10 ml vials) which are earlier labelled with the child's designated code number corresponding to that of the questionnaire filled, plus the letter "A" or "B" indicating prevaccination or postvaccination blood sample

respectively. These were then kept in a freezer at  $-20^{\circ}\text{C}$  until the day the discs are eluted and tested in pairs.

The whatman filter paper disc used here is assumed to absorb 0.1 ml of serum. In each of those individual vials containing a single disc soaked with sample blood is dispensed 1.0 ml of dilution buffer AP\* and eluted overnight without agitation in a fridge at  $+4^{\circ}\text{C}$ . The fluid obtained as such was taken as a 1:11 dilution to the serum.  
AP\* = Alkaline phosphatase

#### ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Different researchers have employed different immunoassay procedures for quantitation of antibody.

The immunoassay technique employed in this study is the ELISA (Appendix I). The choice is made because the ELISA is said to be much more sensitive than the HI (Heamagglutination inhibition) which is the most frequently employed and reported technique (22, 23).

Without any further procedure virus-specific Ig G antibody detection was attempted on this 1:11 prediluted serum. The test plate employed consisted of 6 strips in a special holder. Each strip had 2x8 reaction wells. One row in each case was coated with antigen and the other with control antigen. Antigen and control antigen, negative, were obtained from human cell cultures infected and not infected respectively with measles virus and inactivated

before coating. These were "U" bottomed disposable polystyrene plates manufactured by Behringwerke AG, Marbury, Federal Republic of Germany.

The protocol for the Ig G test was designed in a 1:44 dilution as per the company's recommendation for quantitative/qualitative assay. In order to obtain this dilution, a 0.15 ml (150  $\mu$ l) of dilution buffer AP was dispensed into each reaction well. Then 0.05 ml (50  $\mu$ l) aliquotes of the sera prediluted 1:11 were dispensed with a micropipette into the reaction well coated with antigen and control antigen respectively. These were accomplished in pairs. Measles control serum, positive, human, was added in each plate, at the test dilution to determine the validity. The test was valid when the positive control exhibited a positive reaction in the test dilution. This done, the plate was incubated for 1 hour at + 37<sup>0</sup>c in a moisture chamber. At the end of the prescribed period the plate was removed and the serum dilution sucked off. Washing is done by pipetting at least 0.2 ml (200  $\mu$ l) of diluted washing solution into each well and sucked off after about 1 to 2 minutes while repeating the process twice.

The second step entailed the addition of 0.05 ml (50  $\mu$ l) diluted (1:60) enzyme conjugate solution to each well and incubated for 1 hour at + 37<sup>0</sup>c in a moisture chamber. At the end of the prescribed period the plate was removed and the serum dilution sucked off. Washing is done by pipetting

at least 0.2 ml (200  $\mu$ l) of diluted washing solution into each well and sucked off after about 1 to 2 minutes while repeating the process twice.

On the third step 0.1 ml (100  $\mu$ l) of substrate solution was dispensed to the reaction wells which were then kept in a moisture chamber for 30 minutes at + 37 c. Finally, when the prescribed time was over, the enzyme reaction was stopped adding 0.05 ml (50  $\mu$ l) of stopping solution (2 NaOH) to each well. The intensity of the yellowish - green colour produced by the enzyme substrate reaction was measured in the test plate with a help of a multiskan photometer at a wavelength of 405 nm and the reading expressed as an optical density (OD). A positive reaction is revealed by a difference in absorbance of the antigen and control antigen wells. A value less than or equal to 0.2 OD was assessed as positive. Out of the 341 pairs of blood specimens collected 34 gave an unspecific reaction in the control well reducing the effective laboratory specimen analyzed to 307 pairs. This interference could be attributed to contamination.

#### DATA COLLECTION

Nurses working in those health institutes chosen as a study area and particularly involved in the EPI are recruited, oriented and supervised to perform the vaccination, drawing blood sample and filling the questionnaire (Appendix II). Disposable materials are used

for all procedures. For the EPI eligibles age group, the first blood sample (prevaccination) was taken prior to vaccination which then is subsequently administered on the same day. The vaccine is a further attenuated schwarz strain vaccine (MORBLIVAX by Sclavd S.P.A. Italia). After appropriate dilution a 0.5 ml dose was given subcutaneously on the left upper arm to all of the eligible children. The first part of the questionnaire was administered simultaneously at this encounter. The second part of the questionnaire was completed four weeks later on the second visit when also a second blood sample (postvaccination) was taken. In the second group of children who are below 9 months a blood sample was taken only once and their ages was determined.

## VI LIMITATION OF THE STUDY

When reviewing the monthly EPI attendance record for each clinic, prior to launching the study, five health center/clinics were considered sufficient to obtain the required sample. In reality the situation was far from what was expected and demanded a change in strategy. Therefore 13 other clinics who run nutritional rehabilitation clinics ( NRC) were selected and included to be covered by a mobile team. Health personnel working in those selected clinics perform the screening of children that are eligible for the study and give them appointments on the day when the mobile team is to visit. The total number of children obtained for prevaccination sample collection and measles immunization were 366. Among this 25 dropped out between the first blood sample collection and the second thereby decreasing the effective study population to 341. The reasons for dropout were:

1. Wrong addresses filled on the questionnaire so the child's whereabouts could not be traced.
2. Change of residence without leaving a forwarding address.
3. Temporary absence at the time of visit by the mobile team (vacation, visit .... etc.)
4. Death in one case.

Tracing of defaulters was a formidable task. The team who did the tracing took the postvaccination blood sample at the spot whenever the child was available. Ordering and receiving the laboratory reagents from abroad was difficult and took longer time than was anticipated and this in a way compromised the deadline for the completion of the study.

## VII RESULTS

The summary of the results obtained from the study are presented in the following manner.

Tables I to III deal with the seroconversion of vaccinees. There were a total of 207 children who were found to have a negative (photometric reading less than 0.2 optical density ) Ig G ELISA titer when their prevaccination immunological status was analyzed. Out of these 207 children who were seronegative, 198 (95.7%) had seroconverted when their postvaccination blood sample was tested 28 days after the administration of measles vaccine. 9 (4.3%) didn't show any appreciable rise in their antibody titer that could be considered protective.

Tables IV and V present those results obtained for the 100 children who were found with positive prevaccination titer (photometric reading greater or equal to 0.2 optical density). The rise in antibody titer four weeks after vaccination was analyzed by sex and nutritional status.

Tables VI and VII deal with the history of measles as stated by the mother/guardian . The validity of the history is tested in reference to the prevaccination immunoassay result obtained from those same children. Also the reporting of natural measles is analyzed by the different age categories created for this study.

Tables VIII, IX and X show the incidence of reporting of possible adverse effects, as noted by the mother/guardian who brought the child to the clinic, four weeks after getting the measles vaccine. The distribution by age and nutritional status is presented.

Table XI shows the immunoassay results of the second study group of 100 children that are less than nine months of age. Five of the specimens analyzed show an unspecific reaction reducing the effective sample to 95.

TABLE I<sub>A</sub>

## SEROCONVERSION BY NUTRITIONAL STATUS

NUTRITIONAL STATUS	POSTVACCINATION		TOTAL
	TITER		
	< 0.2	≥ 0.2	
≥ 80	5 (4.7%)	102 (95.3%)	107 (100%)
< 80	4 (4.%)	96 (96.0%)	100 (100%)
TOTAL	9 (4.3%)	198 (95.7%)	207 (100%)

=====

Table I<sub>A</sub> shows the seroconversion of those 207 children who had a negative prevaccination Ig G titer. There is no clinically significant difference in seroconversion rates between the well nourished and malnourished. Further breakdown of the malnourished group gives similar results (see Table I )

TABLE I<sub>B</sub>

## SEROCONVERSION BY NUTRITIONAL STATUS

NUTRITIONAL STATUS	POSTVACCINATION TITER		TOTAL
	< 0.2	≥ 0.2	
≥ 80	5 (4.7%)	102 (95.3%)	107 (100%)
60 - 79	4 (5.1%)	75 (94.9%)	79 (100%)
< 60	0 ( - )	21 (100%)	21 (100%)
TOTAL	9 (4.3%)	198 (95.7%)	207 (100%)

=====

Table I<sub>B</sub> shows the seroconversion of those 207 children who had a negative prevaccination Ig G titer. Looking at this table it can be clearly observed that there is no clinically significant difference in seroconversion rates between the well nourished and moderately undernourished group as well as severely undernourished children who received the vaccine.

TABLE II

## SEROCONVERSION BY AGE CATEGORY

AGE CATEGORY (mos)	POSTVACCINATION TITER		TOTAL
	< 0.2	≥ 0.2	
9 - 11	5 (3.9%)	124 (96.1%)	129 (100%)
12 - 23	4 (5.1%)	74 (94.9%)	78 (100%)
TOTAL	9 (4.3%)	198 (95.7%)	207 (100%)

Table II shows the seroconversion by age category. There is no clinically significant difference in the rate of seroconversion between those that are below 12 months of age but above nine months and those between 12 - 23 months.

TABLE III

## SEROCONVERSION BY SEX

SEX	POSTVACCINATION TITER		TOTAL
	< 0.2	≥ 0.2	
FEMALE	3 (3.6%)	81 (96.4%)	84 (100%)
MALE	6 (4.9%)	117 (95.1%)	123 (100%)
TOTAL	9 (4.3%)	198 (95.7%)	207 (100%)

Table III shows seroconversion by sex. There is no difference in seroconversion titer of the two sexes.

TABLE IV  
RISE IN TITER OF SEROPOSITIVE  
CHILDREN AFTER VACCINATION  
BY NUTRITIONAL STATUS

NUTRITIONAL STATUS	RISE IN TITER (folds)			TOTAL
	<1	1-3	>4	
≥ 80	17	23	5	45
< 80	14	37	4	55
TOTAL	31	60	9	100

=====

Table IV shows the change in antibody titer resulting from vaccinating seropositive children. Out of a total of 100 children 30 (30%) experienced a decline in their Ig G titers four weeks after vaccination for measles, 61 (96.1%) showed a 1-3 fold rise of their prevaccination titer while only 9 (9%) experienced a booster effect. There is no statistically significant difference existing between the different response categories analyzed ( $P > 0.5, X^2$ ). The results are negatively associated with the prevaccination titer.

Mean titer of those who experienced a decline in their titer	>	Mean titer of those who experienced a 1-3 fold rise in their titer	>	Mean titer of those who experienced a ≥ 4 fold rise in their titer
--	---	--	---	--

The overall mean titer was 0.9290 and for those who experienced a decline the mean titer was 1.3589 for those who showed a 1-3 fold rise it was 0.8442 and for those who had a booster effect their mean was 0.2218.

The expected value in one of the cells is less than 5 (4), which tends to make the chi square value inflated, and lead us to make type I error. However, inspite of this, the  $\chi^2$  calculation reveals no statistically significant difference. Moreover, computation employing the Yate's correction gave a similar value. In general, the rule of "5" is considered a conservative one, and many would agree that the  $\chi^2$  test is robust. Perhaps more important, examination of the raw figures show no clinically significant difference.

TABLE V

RISE IN TITER OF SEROPOSITIVE  
CHILDREN AFTER VACCINATION  
BY SEX

SEX	RISE IN TITER			TOTAL
	< 1 fold	1-3 fold	>4 fold	
FEMALE	13	28	7	48
MALE	17	33	2	52
TOTAL	30	61	9	100

=====

Table V shows that there is no statistically significant difference by sex in the response of children who were seropositive at the time of vaccination ( $P > 0.05$ ,  $\chi^2$ ). Refer to the chi square statement made for table IV.

TABLE VI

VALIDITY TEST OF HISTORY  
OF MEASLES AGAINST  
PREVACCINATION ANTIBODY TITER

HISTORY OF MEASLES	PREVACCINATION TITER		TOTAL
	$\geq 0.2$	$< 0.2$	
Yes	31	9	40
No	69	198	267
TOTAL	100	207	307

=====

Table VI presents the prevaccination ELISA Ig G titer of the total 307 children as a reference to the maternal history of measles.

The validity test shows that only 31% of the seropositive children were said to have a history of natural measles infection while the true negative rate (specificity) is 95.6%.

The positive predictive value that indicates the proportion of children with positive history who are also seropositive is 77.5%. The negative predictability that is the proportion of children with negative history who are seronegative is 74.1%.

TABLE VII

HISTORY OF MEASLES BY  
AGE CATEGORY

AGE CATEGORY (mos)	HISTORY OF MEASLES		TOTAL
	YES	NO	
9 - 11	11	186	197
12 - 23	29	115	144
TOTAL	40	301	341

=====

Table VII shows that the maternal reporting of measles for the children aged 12 to 23 months is significantly higher than in children between the age of 9 - 11 months ( $p < 0.05, \chi^2$ ).

TABLE VIII    SIDE EFFECTS BY NUTRITIONAL  
STATUS

NUTRITIONAL STATUS	SIDE EFFECTS		TOTAL
	YES	NO	
> 80	26	143	169
< 80	22	150	172
TOTAL	48	293	341

Table VIII shows possible adverse effects noted and reported by the mother/guardian in the four weeks time after the administration of the measles vaccine. The frequency of observation of the complaints do not show significant statistical variation between well nourished and malnourished children ( $p > 0.05$ ,  $\chi^2$ ). A possible adverse effect was defined as any positive response by the parent/guardian to the question, "were there any health problems with your child noted after vaccination?"

In this study the median onset of adverse effects is found to be the 3rd day while the problems lasted for a median durations of 5.2 days. The most frequently encountered complaint in 77% of the afflicted children was fever, with diarrhea, vomiting and rash contributing 41.6%, 25% and 10.4% of the complaints respectively. There was no clinically significant clustering of any of the problems in the different nutritional groups studied.

TABLE IX

## SIDE EFFECTS BY AGE CATEGORY

AGE CATEGORY (mos)	SIDE EFFECTS		TOTAL
	YES	NO	
9 - 11	28	169	197
12 - 23	20	124	144
TOTAL	48	293	341

Table IX shows that there is no significant statistical difference existing in the reported cases of adverse effects between those children who are 9-11 months and those between 12-23 months ( $p > .05$ ,  $\chi^2$ ).

TABLE X

## SIDE EFFECTS BY SEX

SEX	SIDE EFFECTS		TOTAL
	YES	NO	
FEMALE	19	130	149
MALE	29	163	192
TOTAL	48	293	341

=====

Table X presents the distribution of reported cases of adverse reaction by sex. No significant statistical difference exists ( $>0.05, \chi^2$ ).

TABLE XI

PASSIVELY ACQUIRED  
MATERNAL ANTIBODY BY  
AGE CATEGORY

AGE CATEGORY (MOS)	TITER		TOTAL
	< 0.2	≥ 0.2	
0-2	8	28	36
3-5	23	19	42
6-8	17	0	17
TOTAL	48	47	95

=====

Table XI shows the passively acquired maternal antibodies by age group. There is a highly statistically significant difference existing between the different age groups observed ( $p < 0.05, \chi^2$ ).

Out of the total of 95 children who are under 9 months of age 47 (49.5%) had detectable maternal antibodies while 48 (50.5%) had no detectable Ig G in their sera by the ELISA technique. 100% of those children 6 - 8 months tested do not have detectable protective levels of antibody that is acquired from their mothers. Also 28.5% of those in the age group 0-2 months seem to have received no Ig G antibody from their mothers. This gains momentum and reaches 54.8% in the age group 3-5 months. The decline appears to set in at an early age reaching 100% by 6-8 months of age.

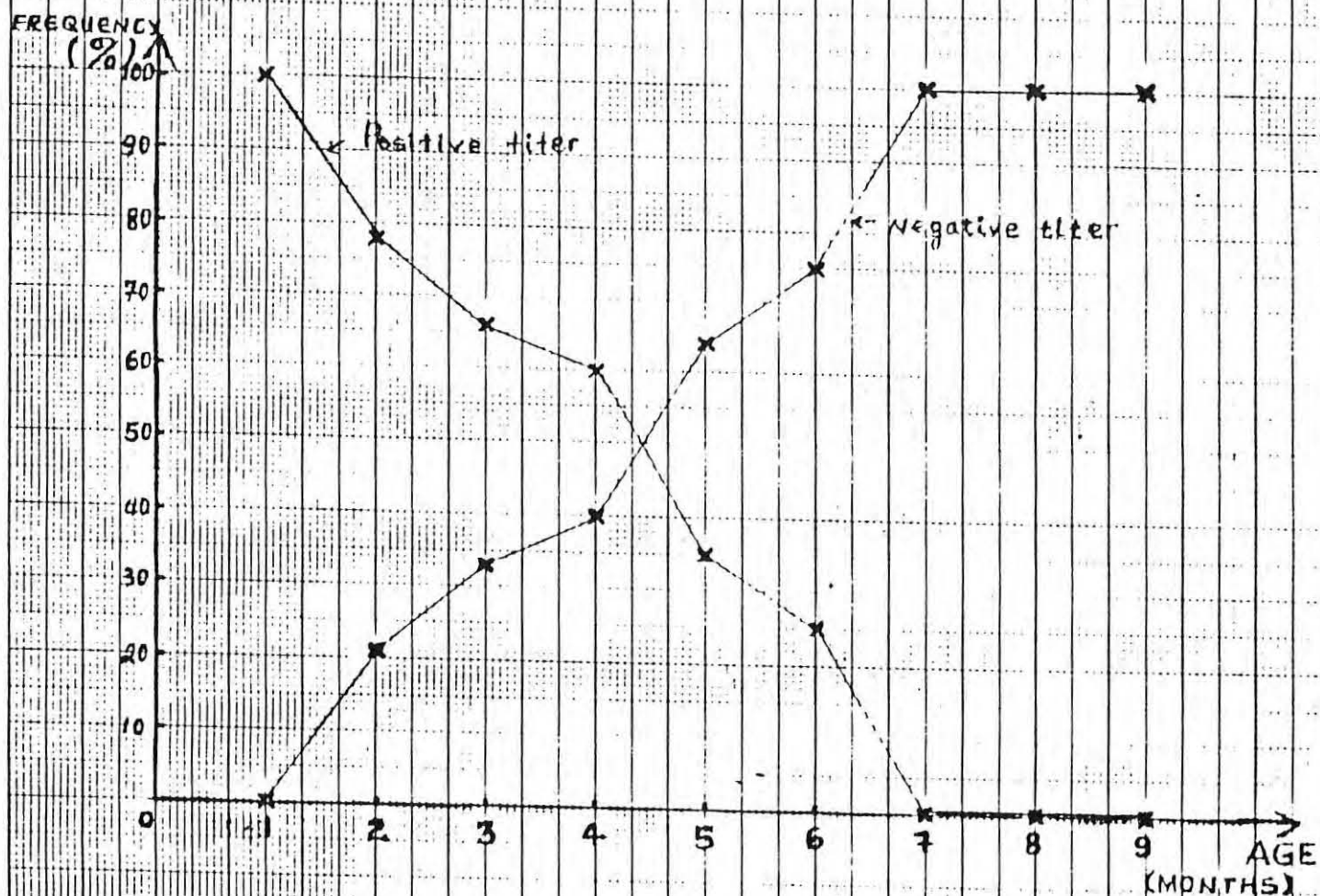


FIGURE 1 - Profile of passively acquired maternal antibody by age in months (0-9)

Figure 1 is a graphical presentation of the findings in TABLE XI. Almost all children are protected at birth but the decline sets at an early age. The proportion of children with a negative titer and are thereby unprotected steadily gains momentum with each passing month reaching the 100% mark at about seven months.

## VIII D I S C U S S I O N

In this paper is presented a brief account of measles, its vaccination and malnutrition with a description of the study objectives, methodology employed and results obtained. Antibody response to further attenuated live measles vaccine was studied in 341 Addis Ababa children 9-23 months of age. Also another group of 100 children who are less than nine months old were studied for passively acquired maternal antibodies. A variety of antibodies are believed to be produced when induced by the measles virus and the most frequently measured include neutralizing (Nt), hemagglutination inhibition (HI), and complement fixation (CF) antibodies (24). The most commonly encountered laboratory technique in the literature for measles antibody detection is the HI, but here ELISA is used which is a much more sensitive laboratory technique.

Out of the 341 pairs of blood specimens analyzed 34 (10%) had an enhanced colour reaction in their control wells indicating that the reaction didn't come from specific antibodies but an interference which presumably might have come from contamination. The effective laboratory samples finally interpreted are 307 pairs.

The optical density obtained on photometric reading is interpreted as per the company's recommendation (see Annex I). A positive reaction is revealed by a difference in absorbance between the antigen coated well and the control

well. A result  $\geq 0.2$  optical density is assessed to be positive and also considered the lowest protective level of antibody titer.

There were 207 (67.4%) children who were found to have a prevaccination ELISA Ig G titer that is less than 0.2 optical density and assessed as a negative reading. The remaining 100 (32.6%) children had a positive prevaccination Ig G titer, that is a reading  $\geq 0.2$  optical density. The discrepancy noted between the negative antibody results obtained for those 6 to 9 months children and the 100 children above 9 months who turned up seropositive can be explained by a seroconversion resulting from natural measles infection or by the inadvertent inclusion of children who had been vaccinated and lost their cards in the study group. However, the inadvertent inclusion is quite unlikely, as any child older than 9 months who had no card, was presumed to have not received any vaccinations. He was therefore given multi-dose vaccines (usually DPT and measles) and was thus excluded from this study. The children are recruited for the study when coming to the study sites (health units) for immunization or from those attending the nutrition rehabilitation clinic when they are eligible for EPI.

A schwarz strain measles vaccine was administered to all 341 children. Of the total 207 children with a negative prevaccination Ig G titer 198 (95.7%) had seroconverted 4 weeks (28 days) after vaccination.

The result is attained under controlled field conditions with a well established cold chain system, trained and supervised vaccinators and a potent vaccine. This markedly high seroconversion rate achieved is consistent with another study which found a 92-100% seroconversion rate when immunization is started after seven and a half months of age in Kenyan children (25).

Different authors have shown variability in seroconversion rates at all ages (26). In this study there was no clinically significant difference in the seroconversion rate observed between vaccinees that are 9-11 months and those above 12 months. Among those 9-11 months, 96.1% had seroconverted on the 4th week after vaccination, a finding that is compatible with other studies done in Kenya, Nigeria, Cote d'Ivoire, Brazil and Costa Rica (26,27). The 12-23 months age group had a 94.9% seroconversion rate and results similar to this were reported by Mandara & Remme<sup>28</sup> and in a review and commentary made by Linnemann<sup>29</sup>. This satisfactorily high seroconversion rate obtained in the 9-11 months age group is explained by an early waning of maternal antibody as is the finding in this study which causes no interference.

There was no clinically significant difference existing in the seroconversion rate of the children that are moderately or severely undernourished from those that are well nourished. Several investigators have observed that malnutrition has no influence on the immune response of vaccinees (29,30,31). The primary immune deficiency in malnourished individuals is a decrease in the ability to produce a cell mediated response so the B cell compartment remains normal and the level of serum immunoglobulins during malnutrition is comparable to those of well nourished individuals (10). The results in this study can be explained in light of the above stated immunological analysis and further supports the efficacy of the measles vaccine in producing immune response in malnourished children. Further analysis of the seroconversion by gender revealed that children exhibit no significant difference in seroconversion rate between males and females.

One can readily conclude from the study finding discussed above that measles vaccine has a highly satisfactory immune response in those above 9 months of age and could be administered regardless of sex and nutritional status.

When measles virus specific Ig G is detected in a sample the assumption will be a prior infection or an active/passive immunization. In this study when analyzing results of the prevaccination blood sample of those 9-23 months my conviction is that they have lost all maternal

antibodies by the time they reached age 9 months. All children who had been vaccinated in the past are excluded from the study after checking their immunization cards. So, apart from those very few cases who might have been included in the study because they have lost their immunization cards all the others are seropositive from infection. These 100 seropositive children were analyzed for the effect of active immunization and their response.

It was found that 9 (9%) children experienced a four fold rise of their postvaccination antibody titer while 91 (91%) didn't show this effect. On further analysis of these results those who didn't experience a booster effect are grouped into those who showed a 1-3 fold rise and those who experienced a decline of their preliminary titer (see table IV). There was no statistically significant difference by nutritional status ( $p > 0.05, \chi^2$ , table IV). The mean titer of those who experienced a decline is much greater than the other two categories. It was found to be 1.3589 while for those who showed a 1-3 fold increase and those who boosted the mean titer is 0.8442 and 0.2218 respectively. Mandara and Remme explained the 6.7% booster finding in their study as a laboratory variation with a four-fold drop of 6.8% on the other end. Also the elevated percentage is justified by a possible additional effect of biological variation. In this study it was also found that there were 10% of the cases with a four-fold drop. The percentage of children experiencing an antibody rise was

equal to those experiencing a four-fold drop. But there was a negative correlation between the mean titers which indicated that the response of seropositive children to vaccination depends on their existing prevaccination level of antibody titer. Those marginally protected will experience a booster effect while those with very high titers show a decline. Possible explanation given to the decline noted is that antibodies are utilized in fighting the vaccine.

For all practical purposes we should determine the lowest protective level of antibodies. ELISA as a seroepidemiological technique for screening purposes is not feasible. Discerning those who are already seropositive will actually help us to curtail unnecessary cost and effort incurred in vaccinating already immune children. To achieve this objective one needs to devise a simple and sensitive screening method.

In vaccinating against measles it is intended to vaccinate only those children who are not immune against the disease. Availability of a simple screening test to identify the non-immune and to exclude the children with immunity due to previous measles infection would save the money spent on vaccinating children already immune to infection (33). One such type of screening in measles is maternal history. In Tanzania, maternal history was used as a screening test in measles immunization. In USA the ACIP (Advisory committee for Immunization Practices) developed a

historical criteria for detecting susceptibles to measles, it accepts physician verified history of measles infection as one component (34).

The validity of maternal history with reference to the prevaccination Ig G titer was checked and the results shown in table VI. In this analysis the laboratory results of those children only above 9 months of age is used in order to avoid interference from maternal antibody in the interpretation. It was found that this screening method has a very low sensitivity calculated at 31% but significantly high specificity found to be 95.6%. This shows that the true negatives are easily picked. If used in actual screening it will serve its purpose in getting those who are seronegative for vaccination while omitting only 4.4% of those who definitely require immunization. But because of its low sensitivity we will be obliged to immunize a significant proportion (69%) of those who are already protected from the disease. So it will not help us much to identify all those who do not require the measles vaccine but make us lose 4.4% of our target population. The positive and negative predictability rates are 77.5% and 74.1% respectively.

There is a statistically significant difference observed in measles history reporting in the different age categories ( $p < 0.05$ ,  $\chi^2$  table VII). This shows the high incidence of measles in the second year of life. Age

specific incidence during an epidemic in a refugee camp had the highest number of cases identified in the age group 1 to 2 years (45).

Fully immunizing a child in the developing world against the six EPI diseases costs \$5 to \$15 (US) (35). We should be cautious and see that some cost reduction strategies might jeopardize the whole purpose of the immunization program. Although cost-effectiveness requires due attention, immunization programmes are considered inexpensive when compared to the cost of treating a case who contracted one of the vaccine preventable ailments. This argument could be extended to include the lives lost, and the psychological and social burdens incurred as a result of the disease. Therefore the findings with the maternal history doesn't tip the balance to be of service and help us in reducing the cost. It may even be hazardous in that it leaves a 4.4% unprotected target group which may add up with similar percentage of cases from vaccine failure which will boost the figure and maintain the endemicity and possibly lead to epidemics. Though the maternal history is not sensitive it may be useful in morbidity surveillance of the community. The sensitivity might be improved by limiting and shortening the recall period.

A study with unvaccinated or placebo vaccinated control children and a frequent follow-up and evaluation by a physician is mandatory to reach a reliable risk

association. In this study the maternal/guardian's report of what is noted during the four weeks after vaccination is taken to obtain a profile of what is going on. There were 48(14.%) children out of the 341 vaccine recipients in this study who turned up with complaints of one sort or the other. About 5-15% of vaccinees are said to develop fever and/or rash<sup>38</sup> but Benenson<sup>39</sup> raises the figure to 30%. Severe complications are rare with measles vaccination (37). In this study undernourished children had no more adverse reactions than well nourished children, table VIII. McMurray et al<sup>40</sup> studied Colombian children and Ifekwunigwe et al<sup>31</sup> on Nigerian children reported no major differences in adverse reaction between malnourished and well nourished children to measles vaccine. So measles vaccine is safe to administer to malnourished children. Further, in this study it was found that there was also no significant difference in the occurrence of adverse effects between the different sexes and between those who are 9-11 months and 12-23 months of age ( $p > 0.05$ , table X and  $p > 0.05$  table XI).

While trying to scrutinize the state of adverse effects in vaccinees we should also consider the background rates which are believed to inflate the actual figures. Most reactions occur at some low but finite rate in an unvaccinated population and are usually of unknown cause (42). However allowing a margin for those background illness rates of unknown etiology and some coincidental problem occurrences the adverse effects are far from

alarming. Another point is the concept of "balancing risks". Here, we are advised to try and see the rates of all these problems to which the vaccine is incriminated and compare them to their counterparts resulting from natural infection. Also the human, psychological and economic components can be brought to the picture. This study does not attempt to look at possible long term effects of measles vaccination, such as subacute sclerosing panencephalitis.

These findings are believed to serve two purposes. One is that we should orient health professionals and encourage them to give vaccines to malnourished children without any reservation. Considering the high mortality of undernourished children from measles the immunization can be considered an indication of first priority. Secondly this knowledge will help particularly people working with the EPI to understand their position clearly and also teach mothers what they should expect. After all, the mothers have a right to know what is going on with their babies.

There are five classes of immunoglobulin and among them only the Ig G class is capable of transplacental passage. Mothers pass this immunoglobulin to the fetus providing it with a shield of specific immune defense in early infancy. This is the period while the baby's own lymphoid system is slowly getting underway. This is how children acquire their protection from measles infection in

early infancy. Determination of the duration of protection of this passively acquired immunity is done by assessing the level of specific antibodies in the blood by immunoassay techniques. In the case of measles antibody, there is a gradual decline throughout the first year of life. This protection is lost as the acquired antibodies are utilized by combination with antigen or catabolized in a normal way. A probable explanation for the high incidence of measles in the first few months of life in developing countries is the early waning of this passively acquired maternal antibody.

The presence of maternal antibody is not important only for protection but also has an implication on response to vaccines. It has a blocking effect on measles immunization when present in the blood at the time of vaccination.

N.A. Halsey<sup>26</sup> stated in his paper that infants from developing countries respond better to measles vaccine than do infants in developed countries.

In this study it was found that only 49.5% of the children less than 9 months old had a protective titer ( $>0.2$  OD) (see table X). This leaves a significant population, around 50.5% in this age group, who are left unprotected and therefore susceptible. The waning sets in at an early age reaching 100% between 6-9 months. The mother/guardians were interviewed to see if the child had contracted measles in the past but none were found in this

group. In a similar study done in Addis Ababa using also the ELISA technique Tadesse and Georgis<sup>46</sup> found, out of the children under 9 months tested, 9.5% were without a protective level of antibody and 6(11.3%) were children who were less than 3 months of age . A passively acquired maternal antibody study in Zimbabwe<sup>43</sup> also using the HI (Haemagglutination Inhibition) technique showed 68% of the children below 9 months to be susceptible and in Tanzania<sup>33</sup> from 6 months onwards almost all antibody titers are not detectable anymore at a dilution of 1:6 (which is far below the protective value of the HI antibody titer). 3% of Zimbabwean children whose age was below 3 months had no detectable measles antibody while 98% of mothers and 97% of cord blood samples were seropositive. However, Broor et al<sup>44</sup> reported 100% of mothers and cord sera positive for measles antibodies.

The explanation for this early waning of maternal antibody could be attributed to children whose mothers had never been exposed to natural measles or vaccination, low persistence of measles antibodies in the mothers, loss of the maternally acquired antibodies at a faster rate due to malnutrition (catabolism), defense (strength a faster rate of causes) or some unexplained genetic factors and children might have received a low level of maternal antibodies (11,13).

This finding has a substantial implication for the country's national expanded programme of immunization in

that one criterion to determine the optimum age for immunization is the time of waning of maternal antibodies which interferes with active immunization and so with seroconversion. The age-specific incidence must also be taken into account and preferably also the age specific case fatality rate (32). It has been documented that most children in developing countries get measles at an age when the case fatality is very high due to malnutrition and current infections (26). Freij et al also had concluded similarly from his hospital based study in Addis Ababa. Community based disease surveillance is essential in order to determine the actual age-specific incidence.

The compromise reached and recommended by WHO at present, to give measles vaccine at the age of 9 months, is only temporary. As the force of measles infection declines we may be forced to adopt a later age for measles vaccination.

## IX SUMMARY CONCLUSION

Measles antibody study was done using the ELISA technique in two groups of children. These were 341 kids aged 9-23 months and a second group of 100 infants who were 0-9 months.

In the first group of 341 children only 307 pairs of blood specimens were analyzable and out of these 207 (67.4%) had a negative prevaccination titer while the remaining 100 (32.6%) were discovered to be seropositive. Vaccine administered to those who were seronegative and above 9 months of age resulted in 95% seroconversion rate. There was no difference observed in immune response to measles vaccine between those undernourished as compared to the well nourished children. Booster effect was not observed in any significant proportion among the seropositive children who received the vaccine. Actually what has been observed could be attributed to a regression to the mean effect.

No particular untoward effect to measles vaccine was noted with the malnourished children as compared to the well nourished. There were no peculiarities found in the clustering and severity of adverse reactions in the different nutritional categories. Waning of maternal antibodies sets in early in life soon reaching 100%. The susceptibles under the age of 9 months are estimated at about 50%. History of measles, in the study population, was

obtained from the mothers/guardians. Maternal history was found to be an insensitive procedure when validated against the prevaccination titer result. The sensitivity for screening is calculated to be 31%.

From this study measles vaccine was found to be safe and affective in moderately as well as severely undernourished children. This should be emphasized in the national EPI policy for immunization. Health professionals should be made aware and as well encouraged to educate mothers of the vaccine side effects expected to occur in a small proportion of the vaccinees. Complete vaccine coverage and particularly measles antigen coverage should be widened to reach the susceptible population before they contract any one of the vaccine preventable diseases. Maternal antibody profile in this study indicates that the child is unprotected by the time he reaches 9 months of age.

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## X I G L O S S A R Y

Age = As determined by the mothers/guardians statement

Antibody = Is a specific substance produced as a reaction to the presence of an antigen; they are associated with certain globulin fractions of the plasma proteins.

Booster effect = A four-fold rise in the postvaccination ELISA titer over the positive prevaccination titer.

Defaulter = One who fails to appear within one week of the appointment date for postvaccination blood collection and administration of the second part of questionnaire.

Dropout = One who never turns up for postavaccination blood collection.

Fever = A rise in temperature of the child as determined by the mother.

Field supervisors = Health professionals who are directly involved in the EPI programme of the Ketana and are working as the research team.

History of Measles = Is an occurrence of measles in the past presenting in a child as a constellation of fever, rash and conjunctivitis and described as such by the mother/guardian.

Ig G (Immunoglobulin G) = Is one of the five classes of immunoglobulins which is most abundant in the blood and the only one that crosses the placenta.

Measles (Syno) Rubeola, Morbilliform.

Pair = Two corresponding prevaccination and postvaccination blood samples taken at a minimum interval of 4 weeks (28 days).

Prevaccination = Period prior to the administration of measles vaccine.

Postvaccination = Period after the administration of measles vaccine.

Seroconversion = Development of a positive titer after vaccination in a previously negative case.

Side effect = Adverse reactions that occur as a consequence of administering a measles vaccine.

Vaccinator = A nurse who had taken a low level EPI training and working with the research team.

### Preparatory work

**Test plate:** Remove from the package, label and allow to stand for approx. 5 min at +20 to +25 °C

**Measles Control Serum, positive, human, for Enzygnost:** Dissolve in accordance with instructions, then dilute the 1:5 solution obtained outside the test plate with dilution buffer AP in such a manner that a log 2 step below the mean titer may be tested. Testing of the exact titer dilution should be recommended since the test is possible only at + one log 2 step.

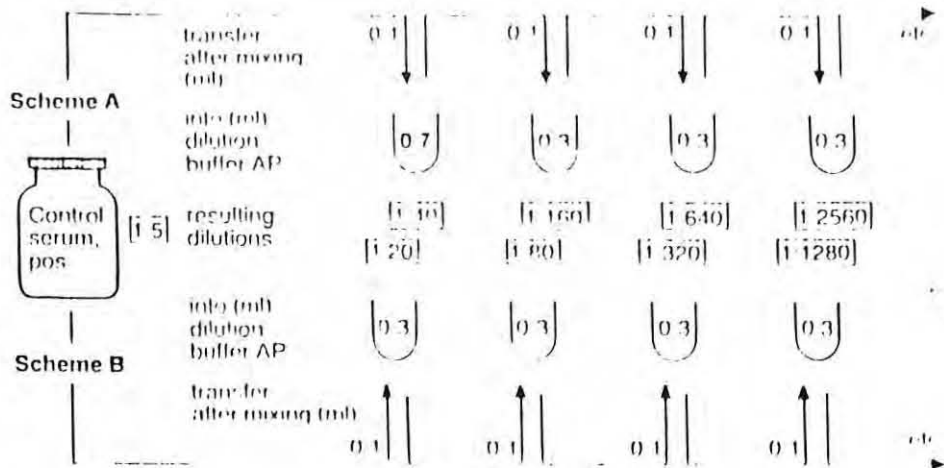


Fig. 1: Dilution schemata for control serum, positive, outside the strip

The desirable end-dilution depends on the IgG (or IgM) titer of the control serum and the amount of dilution buffer AP in the reaction wells of the test plate.

Sample calculation: Given titer 1:2560, serum dilution to be tested 1:1280; end-dilution to be prepared 1:320. Since 1 µl of the test (0.05 ml) is transferred into reaction wells containing 0.15 ml of dilution buffer AP, i.e., select Dilution Scheme B with three dilution steps.

**Anti-Human IgG (or IgM)/AP Conjugate and the Supplementary Reagents for Enzygnost:** Prepare as required in accordance with the respective package inserts.

**Patient serum:** Predilute 1:11 for the IgG test by adding 0.02 ml of patient serum or plasma to 0.2 ml of dilution buffer AP, and mixing. Complement inactivation (30 min at +56 °C) should not have been previously carried out on these samples. For the IgM test after removal of rheumatoid factors see under Possible sources of interference.

**Caution:** All reagents such as dilution buffer AP, enzyme conjugates AP in the dilution employed, and the substrate solution must be brought to +20 to +25 °C before use.

### Procedure

**Immunity status:** Qualitative detection of virus-specific IgG antibodies in a 1:44 dilution.

**Recent Infection:** Qualitative detection of virus-specific IgM antibodies in a 1:42 dilution. Rheumatoid factors (see Possible sources of interference) should be removed in advance.

1. Introduce 0.15 ml of dilution buffer AP into each reaction well (see Fig. 2). This is to be dispensed with when testing for specific IgM antibodies after removal of rheumatoid factors.
2. Pipette 0.05 ml in each case of the sample prediluted 1:11 into a reaction well coated with antigen and control antigen respectively.
3. Incubate the test plate for 1 hour (+5 min) at +37 °C in a moisture chamber. The plate should not be placed on a surface which is a good conductor of heat (metal, moist paper). An empty holder is suitable.
4. Suction off the serum dilutions, and pipette at least 0.2 ml of diluted washing solution into each well. After about 1 to 2 min, suction off, and repeat this washing process twice. Allowing the washing solution to stand for the prescribed period prevents test unspecificities.
5. Add 0.05 ml of the diluted enzyme conjugate solution to each well, and incubate the plate for 1 hour (+5 min) at +37 °C in a moisture chamber as described under 3.
6. Suction off the enzyme conjugate, and wash the plate as described under 4. Add 0.1 ml of substrate solution to each well, and incubate for 45 min (+5 min) at +20 to +25 °C or 30 min (+1 min) at +37 °C in a moisture chamber.

7. Stop the enzyme reaction after the prescribed period by addition of 0.05 ml of stopping solution AP (2N NaOH) Caution! Caustic!
8. Evaluate the yellowish-green colour reaction within one hour.

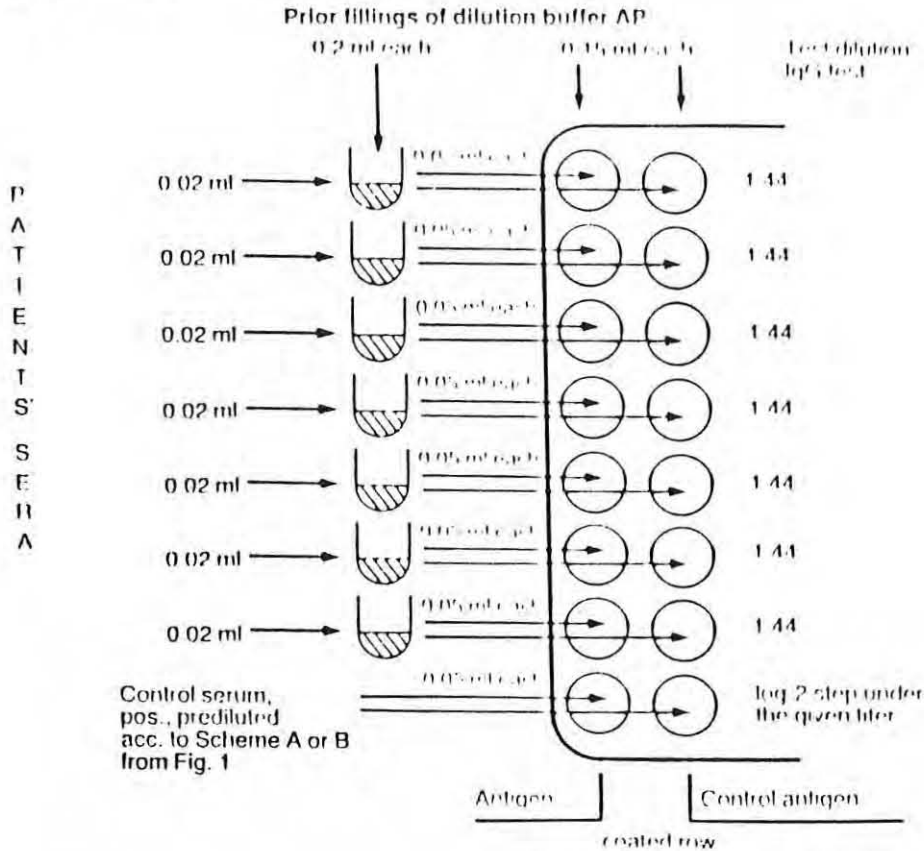


Fig. 2: Predilution and pipetting of the samples to be tested into the reaction wells of the strip.

**Antibody titration:** For the purpose of scientific investigations the virus-specific antibodies may also be titrated. 0.05 ml in each case of a suitable dilution of the patient serum is pipetted into the reaction wells coated with antigen and control antigen respectively and containing 0.15 ml of dilution buffer AP. Titration is effected in log 4 steps by mixing and transferring 0.05 ml in each case to the next pair of wells on the plate. 0.05 ml is to be discarded from the last pair of wells. 1:40 is recommended as the lowest serum dilution for the IgG and IgM test.

Takatsy dilution loops or similar microdilutors and dropping pipettes may not be used.

#### Evaluation

The test results are valid if the control serum, positive, exhibits a positive reaction in the test dilution. On photometric measurement the difference in absorbance attained ( $A_g$  minus Control Ag) with the prescribed test dilution (see Fig. 1) enables accurate data for the record which are suitable for comparison purposes. In the case of the IgM test a 10% absorption should not be undertaken with the control serum, positive.

The test samples may be evaluated as follows:

- a) **Visual:** A positive reaction is present when the intensity of the yellowish-green colour in the well with antigen is greater than that in the corresponding well with control antigen. The positive control antigen serves as criterion in this connection. In the case of the same colour reaction to antigen and control antigen, specific antibodies have not been detected.
- b) **Photometric:** Measurement may be made of a 1:20 dilution of the contents of the well with distilled water in a 1-cm cuvette or directly in the test plate with an appropriate instrument, in both cases at a wavelength of 405 nm against 0.1 ml of substrate solution plus 0.05 ml of stopping solution AP as reference diluted 1:20 or undiluted respectively. On measurement in the test plate the reference solution may be placed in a normal, uncoated microtitration plate with round-bottomed wells for adjustment of the photometer.

When cuvettes are used, all the absorbances measured must be multiplied by 10. This lower factor in comparison with the dilution of the colour solution is the result of the longer path length of the cuvette in comparison with a reaction well.

A positive reaction is revealed by a difference in absorbance

$$A_{\text{antigen}} \text{ minus } A_{\text{control antigen}} > 0.2$$

AA values between 0.2 and 0.3 in the presented test dilution are to be provisionally assessed as positive. If  $AA < 0.2$  on repetition, the sample is to be assessed as negative; if  $\geq 0.2$  positive.

A positive reaction in the IgG test is evidence of a prior infection or vaccination, in the IgM test after exclusion of rheumatoid factors evidence of a recent virus infection.

In the case of antibody titrations above the screening dilution the titer is determined either visually in comparison with the titer of the positive control serum or photometrically by means of an absorbance difference of 0.2 A. The antibody titer is the highest serum dilution at which this difference is just attained.

#### Possible sources of interference

Contaminated or heavily hyperlipemic sera can cause an enhanced colour reaction in the IgG and IgM test.

In the IgM test rheumatoid factors (RF = IgM antibodies to IgG) can lead to false positive results. Any RF which may be present should therefore already be removed during sample preparation for the IgM test. For this purpose a modified method according to Gispert, R. et al. *Clin. exp. Immunol.* 22: 431 (1975) is employed. The manipulation of the RF Absorbent (Code No. OUCG) is described in the package insert, and its effectivity for the ELISA has been confirmed (Ziegelmaier, R. et al. *Laboratoriumsblätter* 33: 19 (1983)). The RF absorption results in a 1:12 dilution of the sample. 0.15 ml of this dilution is introduced in each case into an empty antigen- and control antigen-coated well of the test plate.

Sera treated with RF Absorbent may no longer be used for an IgG determination.

The treatment of sera with RF Absorbent can lead to variations in concentration of the specific IgM by a log 2 step.

Cerebrospinal fluid should not be treated with RF Absorbent, as otherwise false positive results may be obtained. Rheumatoid factors are scarcely to be expected in cerebrospinal fluid.

#### Apparatus

The ELISA is simple to perform, even with a minimum of apparatus.

For the transfer of dilution buffer AP, addition of enzyme conjugate AP, substrate solution and stopping solution AP it is advisable to use a multi-channel pipette with a volume range between 50 and 200  $\mu$ l such as the variable Tetetek Multi-channel Pipette (8 channels with Code No. 77-859-00 and 12 channels with Code No. 77-892-00) from FLOW Laboratories GmbH. The pipettes are accompanied by a set of plastic reservoirs (Code No. 77-824-01) for solutions and buffer, and pipette tips.

For the predilution of patient sera and transfer to the test plate single channel pipettes are to be recommended. The Behring ELISA Washer M15 is well suited to washing of the test plate. It is connected simultaneously to the storage vessel containing washing solution and to a vacuum bottle.

A photometer is not indispensable, but enables an objective evaluation.

All the test procedures (washing, dispensing of reagents and reading with a bichromatic multi-channel photometer) can be carried out automatically by the Behring ELISA Processor.

#### Diagnostic Significance

If a sample contains virus-specific IgG but no corresponding IgM, then a prior infection or an active or passive immunization may be assumed.

A four-fold increase in the titer of the IgG antibodies between two serum samples taken at an interval of 3 weeks and tested at the same time and/or the detection of specific IgM antibodies demonstrates a recent infection.

In the case of sub-acute sclerosing panencephalitis (SSPE) the IgG antibodies are as a rule higher by a factor of 10 to 100 than in the case of a patient recovered from measles.

The Measles ELISA is indicated for differential diagnosis in all infections of unknown origin, in affections of the skin and mucosae for differentiation from e.g. rubella, scarlet fever, serum sickness, drug exanthema, exanthema subitum, and infections by ECHO viruses, and in infections of the respiratory tract and central nervous system.

## Appendix II

CODE NO. \_\_\_\_\_

## QUESTIONNAIRE

DATE \_\_\_\_\_ MONTH \_\_\_\_\_ YEAR \_\_\_\_\_

## 1. IDENTIFICATION

1. Full name \_\_\_\_\_

2. Mothers \_\_\_\_\_

3. Gender \_\_\_\_\_

4. Age (Months) \_\_\_\_\_

5. Ketena \_\_\_\_\_

6. Kebele \_\_\_\_\_ Zone \_\_\_\_\_

7. House Number \_\_\_\_\_

## II. Weight

## III. History of natural measles

Yes \_\_\_\_\_ No \_\_\_\_\_

## IV. Were there any health problems with your child noted after vaccination?

1. If yes, state the type of problem appeared ?

(sign/Symptoms)

-

-

-

2. How many days after the vaccination the problem appeared?

-

3. How long did the problem last?

4. Did you seek any help for this particular health problem?

Yes \_\_\_\_\_ No \_\_\_\_\_

5. If yes Where /Whom?

6. Were you anticipating and ready for such problems.

Yes \_\_\_\_\_ No \_\_\_\_\_

7. If yes, Why?

SAMPLE	DATE TAKEN	DATE ANALYZED	TITER (OD)
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1st Blood Sample

2nd Blood Sample

BOOSTER EFFECT

2nd Titer

1st titer

Name of the person who filled the questionnaire

Title \_\_\_\_\_ Signature \_\_\_\_\_

Presentation of all the possible outcomes of the 366, 9-23 months children obtained.

