



WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

22490

DISTR.: LIMITED
DISTR.: LIMITEE

EPI/GEN/88.11 Rev. 1
ENGLISH ONLY

RESEARCH INTO ALTERNATIVE MEASLES VACCINES IN THE 1990'S

Dr C J Clements, Medical Officer, EPI.
Dr Julie B Milstien, Scientist, BELG.
Dr M Grabowsky, Consultant, EPI.
Dr J Gibson, Consultant, EPI.

This document is not issued to the general public, and all rights are reserved by the World Health Organization (WHO). The document may not be reviewed, abstracted, quoted, reproduced or translated, in part or in whole, without the prior written permission of WHO. No part of this document may be stored in a retrieval system or transmitted in any form or by any means - electronic, mechanical or other without the prior written permission of WHO.

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

Ce document n'est pas destiné à être distribué au grand public et tous les droits y afférents sont réservés par l'Organisation mondiale de la Santé (OMS). Il ne peut être commenté, résumé, cité, reproduit ou traduit, partiellement ou en totalité, sans une autorisation préalable écrite de l'OMS. Aucune partie ne doit être chargée dans un système de recherche documentaire ou diffusée sous quelque forme ou par quelque moyen que ce soit - électronique, mécanique, ou autre - sans une autorisation préalable écrite de l'OMS.

Les opinions exprimées dans les documents par des auteurs cités nommément n'engagent que lesdits auteurs.

1. INTRODUCTION

1.1. Background

Amid mounting concern over young children contracting measles before the recommended age of immunization, there has been a search for a vaccine which could protect children from six months of age or earlier. This paper examines the issues related to research into alternative measles vaccines.

Despite the availability of a safe and effective vaccine for 25 years, measles remains an important paediatric health problem in the developing world, causing over 1.5 million deaths each year. The prime reason for this is the failure to provide most children with measles immunization. As of October 1988, measles immunization coverage in developing countries was only 53%. Since the case-fatality rate is especially high among young infants, it would be highly desirable to immunize as early in life as possible. The vast majority of infants, however, are born with transplacentally acquired passive immunity which protects them from the ravages of early measles disease, but also interferes with live virus immunization early in life. (See Hayden G, : EPI/RD/88.WP4).

In establishing a recommended age for routine immunization, a compromise was therefore reached by WHO (1) between the desire to immunize as early as possible and the desire to maximize the rate of seroresponse. For a specific population, this choice depends on two major factors: the rate of seroconversion among infants at different ages and the risks of measles exposure and illness at these same ages. WHO has recommended measles immunization as soon as possible after the age of 9 months for most developing countries.

1.2. Delayed Impact on Mortality and Morbidity

Measles has been shown to have an effect on mortality and morbidity not only in the period immediately after the acute infection, but also for a considerable period thereafter. The disease may therefore have a greater effect on mortality than was previously assumed. Unfortunately, there are very few studies examining the impact of measles on later morbidity and mortality. In many of the studies the comparison between previous cases and controls is associated with serious methodological problems.

Hull went back 3 and 9 months after an outbreak of measles in a village in the Gambia to assess the impact of measles (2,3). This study indicated a highly significant excess risk of dying after acute infection among former measles patients compared to community controls. Mortality was distributed throughout the 8 months of follow-up. The excess mortality seemed to be particularly high for the children who had contracted measles under one year of age. This is confirmed in a study from Nigeria, where Osagie (4) re-identified 106 cases of measles and 106 controls who had visited a hospital clinic the previous year.

The study found a strikingly higher mortality of 31.8% among the children who had measles before one year of age compared, for example, with the mortality of 3.7% for children who had not contracted measles by their second year of life.

In a demographic follow-up study in Burkina Faso, children who had contracted measles during an epidemic were visited every 4th month (5). The study suggests an excess mortality for at least 4 months after measles among children who had measles before two years of age.

Whereas differences in mortality during the first year after measles infection were observed in these studies, a study from Guinea-Bissau (6) reported excess mortality in the following year as well (Table 1). Children who were not immunized in the beginning of 1980 because they had had measles during the first months of 1979 had a significantly higher mortality during 1980 (i.e. 10-21 months after infection) than the children of the same age group who were immunized in the beginning of 1980. Similar observations of delayed excess mortality more than one year later were made during 1984 after an epidemic in the previous year (7).

TABLE 1.
MORTALITY 10 - 21 MONTHS AFTER MEASLES INFECTION
COMPARED WITH COMMUNITY CONTROLS IMMUNIZED AGAINST MEASLES,
GUINEA-BISSAU (6)

Age at beginning of 1980*	Mortality during 1980		Odds Ratio	95% Confidence Interval
	History of measles in 1979	Controls		
12 - 23 mo.	6% (4/67)	1% (1/128)	8.1	1.2-53.2
24 - 35 mo.	6% (3/51)	2% (2/109)	3.3	0.6-17.6

* Most of the children had had measles in March-April 1979 i.e. 8-9 months earlier.

Several studies have examined delayed morbidity after measles infection. Bhaskaram (8) followed for a period of 6 months, measles cases and controls who had never had measles. Those infants who had contracted measles had 10 times more days of illness than controls; the difference was particularly marked in the first three months after measles.

The available data strongly suggest that previous measles cases have a significant excess morbidity and mortality compared with community controls. Studies to date suggest that the risk of delayed mortality is particularly increased for children who had measles before one year of age. Most studies

have emphasized the period of 1 to 3 or 6 months after infection as the critical one. However, the possibility that the difference in risk may continue for an even longer period has not been sufficiently evaluated. The inferences to be drawn from the above is that it is particularly important to prevent measles in the first year of life, not only because of deaths averted in the acute phase but also in subsequent months.

1.3. Four Possible Strategies to Increase Protection of the Young

Implementation of measles immunization at age 9 months has been successful in many areas in reducing measles morbidity and mortality. In some areas, however, measles morbidity and mortality among 6-9 month-old infants remain substantial (9). In addition, many infants drop out of the vaccine delivery system between the time of completing DPT and polio immunizations at 4-6 months of age and the time for scheduled measles immunization at age 9 months. Markowitz (10) described four ways in which the failure to adequately protect infants with the current vaccine might be overcome:

a) achieving a high enough vaccine coverage so that herd immunity of the older children will protect the younger ones,

b) using seroconversion studies to determine the youngest possible age at which vaccine can be administered in a given population,

c) a two dose strategy,

d) overcoming the problem of residual maternal antibodies by a different strain of vaccine or different route of administration.

While a) has been successful in some countries, medium to high coverage levels have had less impact than had been hoped in preventing outbreaks of measles and in protecting the very young from measles in high population density areas.

Although some countries have attempted b), the age of administration has not been reduced to as low as six months, thereby leaving some susceptible children still unprotected. The remaining strategies, namely the use of alternative vaccine strains and a two dose regime will be discussed in more detail.

2. CHARACTERISTICS OF MEASLES VACCINE STRAINS

2.1. Six Strains

A number of measles vaccine strains are presently in use throughout the world. This paper will concentrate on those strains which are presently under consideration for use in children below nine months of age, or which are useful as benchmarks. These include four strains derived from the Edmonston strain, including the Schwarz and Moraten strains in use in much of the Western world, the AIK-C strain from Japan, and the Edmonston-Zagreb (E-Z) strains from Yugoslavia. Also considered are the Leningrad-16 strain from the USSR and the CAM-70 strain from Japan, which are separate isolates derived by a number of passages. A schematic history of these passages is given in Table 2, and a summary of the Edmonston attenuations can be found in reference (11).

2.2. Method of Attenuation

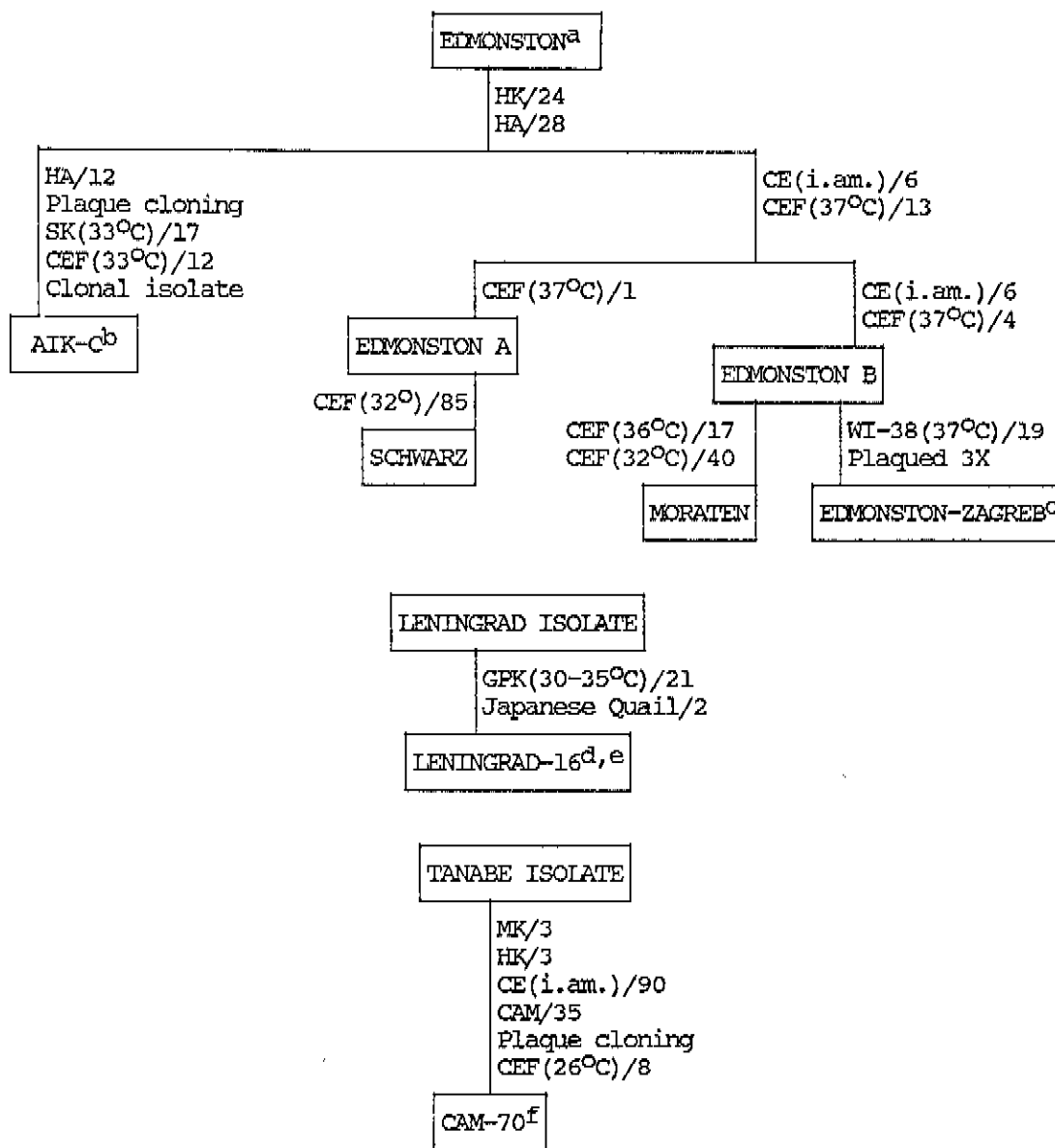
Five of these six strains, Schwarz, Moraten, AIK-C (12), Leningrad-16 (14, 15), and CAM-70 (16), were attenuated by low temperature passages, and three of them were derived from plaqued isolates. The CAM-70 strain was derived from a clone selected by plaque isolation from chick chorio-allantoic membrane at 36°, then passaged eight times in chick embryo fibroblasts at 26°. The E-Z strain (13) was plaqued three times, at the ninth, eleventh, and thirteenth passages in human diploid WI-38 cells, selecting large plaques each time. The AIK-C strain (12) was derived from a plaqued isolate in sheep kidney cells adapted to 33°, and after readaptation to chick embryo fibroblasts, a further clonal isolate was used for vaccine production. Only in the development of the E-Z strain, however, was the large plaque type specifically selected.

2.3. Plaque Morphology

The different strains have characteristic plaques. This is reflected in the fact that there is a difference in the plaque size of measles strain isolates grown on Vero cells (17,20). Schwarz and Moraten strains have small plaques, while E-Z forms large granular plaques. The Leningrad-16 strain forms a majority of large syncytial bordered plaques, as does the CAM-70 strain (G. Mann, personal communication, 1988). Thus, three strains, E-Z, CAM-70, and Leningrad-16, share the characteristic of large plaque morphology on Vero cells.

Ikic et al. (13) reported that the plaques of the E-Z vaccine in HeLa cells were large, with a mean plaque size of 0.61 mm, and uniform in size, while those produced by the Schwarz strain were smaller, with a mean plaque size of 0.49 mm, and of variable size. Thus, in at least two cell types, the E-Z vaccine has

TABLE 2.
ATTENUATION HISTORY OF SOME MEASLES VACCINES



Abbreviations: HK=human kidney cell culture; HA=human amnion cell culture; SK=sheep kidney cell culture; CE(i.am.)=chick embryo (intraamniotic cavity); CEF=chick embryo fibroblast cell culture; WI-38=human diploid cell line; GPK=guinea pig kidney cell culture; MK=monkey kidney cell culture; CAM=chick chorioallantoic membrane. "/number" indicates the number of passages

- a Hirayama (11)
- b Makino (12)
- c Ikic (13)
- d Gordienko (14)
- e Smorodintsnev (15)
- f Okuno (16)

shown large and uniform plaque size in contrast to the Schwarz strain which has a smaller plaque size.

2.4. Growth in Human Diploid Cells

The number of passages in human diploid cells (HDC) required before attainment of optimal titers was studied by Wegman et al (17). The Schwarz strain showed increasing titers on passage, attaining a titer of $10^{4.07}$ after five passages, and $10^{6.15}$ after 20 passages. Similarly, the CAM-70 strain showed optimal titer ($10^{6.0}$) only after 20 passages on human diploid cells, while the Leningrad-16 and AIK-C strains attained optimal titers ($10^{6.0}$ and $10^{5.45}$, respectively) after five passages in human diploid cells. The E-Z strain, having been already adapted to human cells, attained a titer of $10^{5.65}$ on the first passage. Thus, the ease of adaptability to HDC for these five strains decreases in the order E-Z > Leningrad-16 = AIK-C > Schwarz = CAM-70.

According to Whittle et al. (22), the E-Z strain grows more rapidly than the Schwarz strain on both Vero and MRC-5 cells. This may be an important point when commercial aspects of production are considered.

A potentially important biological characteristic of the E-Z vaccine has been recently published (23). These investigators found significant quantities of defective interfering (DI) particles in a preparation of E-Z virus, in contrast to preparations of two other Edmonston-derived strains (Schwarz and Moraten). There has been a great deal of speculation as to the role of DI particles in attenuation, and it is important to evaluate the presence of DI particles in the vaccine preparations in current use. Since the addition of DI particles to a virus infection is known to be one mechanism for establishing a persistent infection, vaccines should be free of DI particles if their presence is not important to vaccine action. Moreover, the absence of DI's should allow higher virus yields.

2.5. Potency Measurements

The WHO Requirements describe two alternative ways of determining the potency of measles vaccine: by measurement of plaque forming units (PFU) in Vero cells, and by determination of tissue culture infective doses (TCID₅₀), also in Vero cells (24,25). However, the requirements do not specify in detail the optimal methods of performing these tests. Comparative studies suggest that the potency measurements may vary depending on the method of determination (Table 3).

For example, using equivalent conditions of virus adsorption (adsorption to dilute cell suspension), Mann (20) found that 1 TCID₅₀ was equivalent to 0.60 PFU in tests on the Moraten strain in Vero cells. This relationship was

equivalent to that theoretically expected (26). When comparing TCID₅₀ assays in which the virus was adsorbed to dilute cell suspensions to plaque assay on preformed monolayers, Kenny and Schell (27) reported that the plaque assay method was ten times more sensitive. Workers in Mexico (28) found a similar observation for the Edmonston-Zagreb strain using similar assay systems. Albrecht (29), using preformed monolayers for both TCID₅₀ and PFU tests, found that the two methods gave similar values for Edmonston-Zagreb. He also found that the TCID₅₀ method was more sensitive than PFU by about 0.4 log₁₀ for the Schwarz strain. These data are summarized in Table 3.

TABLE 3.
COMPARISON OF DIFFERENT METHODS OF POTENCY DETERMINATION

VACCINE STRAIN	ADSORPTION METHOD* (PFU/TCID ₅₀)	PFU/TCID ₅₀	REFERENCE
NA	THEORETICAL	0.69	26
MORATEN	S/S	0.60	31
SCHWARZ	P/P	2.5	36
SCHWARZ	P/S	10	27
EDMONSTON-ZAGREB	P/P	1.0	36
EDMONSTON-ZAGREB	P/S	17	28

* P = virus adsorbed onto preformed monolayers
S = virus adsorbed in dilute cell suspension

Based on the Poissonian distribution of virus particles among cells for the TCID₅₀ assay (26), the theoretical ratio between these two assay methods, is 1:.69. There are many influences on the relationship between these two methods for determination of vaccine potency, including the method of adsorption of virus; cell type and concentration used for the test; effect of overlay and stain in the plaqueing method; design of the test; and influence of tissue culture plates, media, temperature, and humidity. According to Cooper (26), if 70 plaques can be counted per plate, one plaque culture is statistically equivalent to 100 end-dilution hosts (e.g., microtiter wells). Therefore, as the tests are generally performed, the PFU method is statistically more accurate.

The differences and variability in the methods highlight the need for more studies to determine the best way to measure potency. Consideration should be given to expressing potency in terms of an International Standard Measles Vaccine. Further details of this and other technical details can be found in "Research on alternative measles vaccines: technical background and recommended format for reporting field trials", WHO/EPI/GEN/88.12.

Moreover, since it is not known how the growth characteristics of the different measles virus vaccine strains differ relative to each other when grown in different host cells, the cells used for assaying potency must be carefully defined. Potency estimates should be related to clinical efficacy. One possible interpretation of the results obtained on dose dependence of seroconversion in infants under 9 months of age is that the potency of the Edmonston-Zagreb vaccine as determined in the typical Vero cell assay system is greatly underestimated.

2.6. Stability

WHO Requirements (24) specify a thermal stability of a measles vaccine preparation such that the potency does not decrease by more than 1 log₁₀ after incubation of the unreconstituted vaccine for one week at 37°. The performance of a vaccine in this test may depend on the vaccine strain, stabilizer used, and the freeze drying technique. Some average titer losses on one week incubation at 37° are given in Table 4. A study by Mann et al. (31) found that vaccines

TABLE 4.
STABILITY: REPRESENTATIVE TITRES OF VACCINE STRAINS

STRAIN	TITRE OF VACCINE LOG TCID ₅₀	TITRE LOSS 1 WEEK, 37° C
EDMONSTON-ZAGREB, MEXICO	3.65 ^a	0.47-0.63 ^a
EDMONSTON-ZAGREB, YUGOSLAVIA	4.19 ^b	0.7 (11 days) ^c
CAM-70, BRAZIL	4.30 ^d	0.59 ^d
SCHWARZ, UNICEF SUPPLIERS	3.77 ^d	0.24-0.44 ^d
SCHWARZ, BELGIUM, 1988	3.82 ^e	0.02 ^e
SCHWARZ, BELGIUM, 1983	3.94 ^f	0.24 ^f
AIK-C	3.70 ^g	1.0 (4 weeks) ^g

a -Personal communication, Gustavo Kado Boll, Instituto Nacional de Virologia, Mexico, 1988

b -Personal communication, Miroslav Beck, Institute of Immunology Zagreb, 1987

c -Ikic et al. (13)

d -Personal communication, Eduardo Chaves Leal, Instituto Nacional de Qualidade em Saude, Brazil, 1988

e -Personal communication, P.E.Lemoine, Institute d'Epidemiologie et Higiene, Brussels, 1988

f -Andre (30)

g -Personal communication, Akiyama, 1988

able to pass this accelerated stability test (loss of less than 1 log on incubation at 37° for 1 week) would be predicted to be stable for one year at 8°C. This test was included in the WHO Requirements (25).

For the reasons mentioned above, determining the minimal dose required for seroresponse depends on the method used to determine the administered dose. The definition of seroresponse, to be discussed in more detail below, is also an important consideration in determining dose dependence, as well as duration of immunity. With these caveats in mind, some information on the subject is available. Makino (12) has reported that a dose of >100 TCID₅₀ of AIK-C vaccine gave a 100% seroconversion rate in Japanese children eight months to eight years of age. Ten year follow-up (11) shows continuing protection from measles in successfully immunized individuals. One study on the CAM-70 strain (32) showed a minimum dose for 100% seroconversion of 2500 TCID₅₀ in children 9 months to 7 years of age. Follow-up studies (33) showed detectable antibody in all children 12 to 13 years after immunization. Persistence of immunity of the Leningrad-16 strain in the USSR has been demonstrated for 15 years (34) and in Yugoslavia, the E-Z strain has been shown to give persisting immunity for at least 16 years (35). All these studies have been done in children without maternal antibody.

A number of studies on the E-Z strain suggest the dose dependence curve of seroresponse is shifted to lower doses relative to the Schwarz strain in young infants. It may be this phenomenon which enables the E-Z vaccine to overcome the barrier of maternal antibody. However, to date no studies have been performed on duration or dose-dependence of long-term immunity in infants with maternal antibody. Such studies will need to be done. Will the protection afforded by immunization of very young infants be as durable as that of older infants and children? What if the vaccine induces a very adequate rate of seroconversion at age 6 months but the geometric mean titers fall away over time? Encouraging reports from the Gambia indicate that excellent titers are maintained for at least two years (22). Other preliminary reports are not so reassuring (M. Just, personal communication 1988).

3. SEROLOGICAL RESULTS

3.1. Measurement of Serological Response

There are three serological tests in general use for measuring seroresponse after measles immunization: the haemagglutination inhibition test (HI), the plaque reduction neutralization test (PRN), and the ELISA assay. Each of these tests measures a different aspect of measles immunity, that is, antibody response to measles haemagglutinin, infectivity, and viral components respectively. Not unexpectedly, these tests all have different sensitivities. According to Albrecht (personal communication, 1988), the plaque reduction

neutralization test is of such sensitivity that if sera are negative by the test, primary vaccine failure is indicated. This test is 60 times as sensitive as the HI test. A comparison of the use of some of these tests has been published (36).

Under similar conditions, the ELISA test is about as sensitive as the PRN test. However, one characteristic of the ELISA test is that maternal antibody is not well measured by this method. In addition, antibody titer as measured in this way tends to increase over time rather than reaching a plateau or dropping slightly, perhaps because it measures existence of antibody to viral nucleocapsids and other non-infectious viral components as well as to infectious viral particles.

Because of these differing sensitivities, serological data should be reported in comparison with data derived using the international standard human anti-serum. However there will still be variability introduced by the times at which sera are collected.

A second problem in the measurement of serological response is that of the definition of seropositivity. Some investigators have reported results which defined sero-response as a change from no detectable antibody at the lowest dilution used (usually 1:4) to detectable antibody in the post-immunization specimen. Other investigators used a two-fold to four-fold rise from pre-immunization levels. Yet others used sero-positivity with a cut-off point between 50 and 200 milli-international units (m.I.U.) to indicate protective levels of antibody.

To date, the correlation between antibody titer and protection is not completely clear. Nor has the role of cell-mediated immunity in vaccine induced protection been adequately explored. Whether different measles vaccine strains differ sufficiently in surface determinants to be distinguishable in terms of their ability to be neutralized by maternal antibody is another question which deserves further study.

3.2. Completed Serological Studies

The eight published studies of immunogenicity of E-Z measles vaccine (Table 5) have been reviewed by Markowitz and Bernier (10). Sabin, et al, gave E-Z and Schwarz vaccine by aerosol administration to infants 4 to 6 months of age in an attempt to overcome interference from maternal antibody (18). When 90% of infants seroconverted after receiving E-Z vaccine compared to 39% given Schwarz vaccine, attention focused not only on the aerosol route but also on the greater immunogenicity of the E-Z strain. A subsequent study by Sabin, et al, with subcutaneous administration of E-Z vaccine in 4 to 6 month olds showed an overall rate of seroconversion of 84% at 14 weeks using ELISA and PRN assays (36).

The high immunogenicity of E-Z vaccine in young infants has been demonstrated in Yugoslavia by Beck, et al, and in the Gambia by Whittle. (35,41,37). Beck found a seroconversion of 92% in 4 to 6 month olds and 100% in 6 to 12 month olds using either subcutaneous or intranasal routes (35). Whittle used aerosol, subcutaneous and intradermal administration in 5 month olds to obtain a response rate of 88 to 100% (37). Both of these studies used a dosage which was much higher than that in the standard Schwarz vaccine (see Table 5). Favorable results with subcutaneous administration and operational difficulties with aerosol administration have resulted in subsequent research focusing on the subcutaneous route of administration in young infants. However, in principle other routes of administration (aerosol, intranasal, conjunctival) are still of interest to EPI.

A key question is the relative importance of dose and strain in achieving seroconversion at an early age. Will the Schwarz and E-Z vaccines be equally effective at high dosages? Three published studies have directly addressed this question.

1. A Mexican study in 6 to 9 month old infants showed a higher seroconversion rate for subcutaneous E-Z (100%) than for Schwarz (80%), even though the Schwarz was given in a higher dosage (38).
2. In Bangladesh, 4 to 7 month olds were found to have higher seroconversion with E-Z following the subcutaneous route (62% vs 37%) using standard dose preparations but not the aerosol route (35% vs 34%) (39).
3. In the Gambia, subcutaneously administered E-Z vaccine given at 10 000, 20 000 and 40 000 PFU resulted in higher GMT's with higher doses (22). The 40 000 PFU dosage given subcutaneously resulted in positive responses in all infants and higher antibody levels than doses of 20 000 or 10 000 PFU. In further trials, both E-Z and Schwarz were given in the 40 000 PFU dosage. A higher proportion of the E-Z group responded (36/39 vs 16/35).

Results from a clinical efficacy trial from Guinea-Bissau have recently been published (40). High dose E-Z (40 000 PFU) was compared to standard dose Schwarz (6 000 TCID₅₀) in 558 children aged 4 months and older. In a two year follow up, there were no cases diagnosed in the E-Z group and 14 in the Schwarz group; 9 of these cases occurred in children before 9 months of age. Thus the E-Z vaccine provided significant protection against measles both before and after the usual age of immunization.

These studies suggest that the E-Z strain can effectively protect infants from measles at ages earlier than 9 months. It is to be hoped that ongoing and planned studies will continue to investigate the relative importance of strain and dose effects.

TABLE 5.
COMPLETED IMMUNOGENICITY STUDIES OF EDMONSTON ZAGREB MEASLES VACCINE (10).

Study + Location	Age mos.	A/b test	Edmonston Zagreb		Schwarz	
			Dose (route)	% response	Dose (route)	% response
Sabin (18) Mexico	4-6	FN ELISA	12000 PFU (aerosol)	90	12000 PFU	36-39
Sabin (36) Mexico	4-6	FN ELISA	5000 PFU (subcutaneous)	84		
Beck (35) Yugoslavia	4-6	HI	39800 TCID ₅₀ (subcutaneous)	92		
			39800 TCID ₅₀ (intranasal)	97		
Beck (41) Yugoslavia	6-1	HI	25100 TCID ₅₀ (subcutaneous)	100		
			25100 TCID ₅₀ (intranasal)	100		
Whittle (37) Gambia	5	PI HI	3500 PFU (aerosol)	88		
			7000 PFU (aerosol)	97		
			39000 PFU (subcutaneous)	100		
			11400 PFU (intradermal)	100		
de Castro Mexico (38)	6-9	HI	1950 TCID ₅₀ (subcutaneous)	100	6300 TCID ₅₀ (subcutaneous)	80
Khanum (39) Bangladesh	4-7	HI	5000 TCID ₅₀ (subcutaneous)	62	6300 TCID ₅₀ (subcutaneous)	37
			5000 TCID ₅₀ (aerosol)	35	5000 TCID ₅₀ (aerosol)	34
Whittle (22) Gambia	5	PI HI	20000 PFU (subcutaneous)	94-100	20000 PFU (subcutaneous)	76-88
			40000 PFU (subcutaneous)	100	40000 PFU (subcutaneous)	79-84

3.3. Comparability of Results

A summary of the design of eight recent and ongoing studies of alternative measles vaccines is presented in Table 6. There are important differences in the techniques used to measure response to the vaccine in the different studies. Those which use a more conservative definition of response show a lower response rate. The different measurement techniques (ELISA, HI, PRN) may not give similar results. Some studies use seroconversion as evidence of successful immunization while others use a fourfold rise in titre. Such differences limit the comparability of the studies.

As already discussed, comparison is complicated by variations in the potency of the vaccines used, the method of testing for measles antibody, and the criteria used to determine whether an individual had responded successfully to immunization. The technical details of each issue have been raised in preceding paragraphs. Researchers are encouraged to follow the suggestions for biological parameters and the protocol for their presentation and reporting of results (document WHO/EPI/GEN/88.12). Adherence to these recommendations will go a long way to overcome the problems of comparability between studies.

Another point in comparing responses is that every manufacturer's product must be considered a new biological product until proven equivalent to existing products. Thus, on the basis of experience with currently marketed Schwarz strain vaccines, it is fair to consider them as one entity regardless of manufacturer. Most studies on the E-Z vaccine have been done on vaccine produced in Yugoslavia, while Sabin's original studies (18) used the E-Z vaccine produced in Mexico. Preliminary results have shown slight differences in seroresponse between the Mexican and Yugoslav products in the one study where they were directly compared (L. Markowitz, personal communication).

3.4. Efficacy Studies

Only two long term vaccine efficacy studies have been reported. In Guinea-Bissau, 234 infants were immunized with E-Z vaccine at age 4 months and there have been no cases of measles in this group. A comparison group of 235 infants given Schwarz vaccine at nine months experienced 4 cases (40). In a similar

TABLE 6.
STUDIES OF ALTERNATIVE MEASLES VACCINES, DOSAGES AND SCHEDULES, 1988

Site	Strain	Age in months	Serology (assay)
Gambia	EZ-M	4	SP>100 miu (HI)
	SC-S	9	
Guinea-Bissau	EZ-M	4	N/A
	SC-S	9	
Haiti	EZ-M,H	6	SP>400 miu SC>2X or - to + (ELISA)
	EZ-M,H	9-11	
	SC-M,H	6	
	SC-M,H	9-11	
Mexico	EZ-S,H	6	SC>2X or - to + or increase in titre (PRN)
	EZ-Mx,My	6	
	SC-S,M,H	6	
	EZ-S	9	
	SC-S	9	
Senegal	EZ-H	5	SP>100 miu (HI)
	SC-H	5	
	SC-S	10	
Togo	EZ-H	4-5	SP>20 miu or SC - to + (HI)
	SC-H	4-5	
	KI-M	4-5	
	SC-S	8-10	
	KI-M	8-10	
Turkey	EZ-SM	4-7	SC=4x or - to + (ELISA)
	SC-SM	4-7	
Zanzibar	EZ-SM	5-7	SP>50 miu or SC - to + (ELISA)
	SC-SM	5-7	

EZ=Edmonston-Zagreb, SC=Schwarz, KI=AIK-C
H=High (log 5.5), MH=Medium-High (log 4.8), M=Medium (log 4.5),
SM=Standard-Medium (log 3.8), S=Standard (log 3.5)
SC=Seroconversion, SP=Seropositivity
Mx=Produced in Mexico, My=Produced in Yugoslavia
Acknowledgement to Dr. R. Bernier, who compiled this table.

study in the Gambia, there was one case in 119 infants who were immunized with E-2 at 4 months of age and 2 cases in 120 infants who received Schwarz at nine months of age. In these clinical studies, E-2 vaccine given at 4 months gave a response which was as good as or better than the Schwarz vaccine at nine months.

4.0. PRODUCTION AND LICENSING

There are several considerations a manufacturer must keep in mind especially for production of a vaccine which will be sold through international tendering to United Nations agencies. These include the technical aspects of production; the right to produce, distribute, and market a strain; national licensing requirements in the country of manufacture; and requirements of the UNICEF tender for those manufacturers supplying to UNICEF.

4.1. Technical Aspects of Production

If the change in the vaccine is only a change in the virus strain, the manufacturer's problem is to assure optimal conditions for virus growth, once the virus seed has been tested and shown to meet existing requirements for safety and efficacy. This may depend on the attenuation history of the vaccine strain. For example, the AIK-C vaccine has been derived by passage through sheep kidney cells. It may be important in the use of this strain to demonstrate freedom of the seed from adventitious agents found in sheep, for example, lentiviruses.

If the change also involves a change in cell substrate, as, for example, a change to the Edmonston-Zagreb strain produced in human diploid cells, then a working cell bank must be established and tested, consistent with existing requirements.

Other technical issues include consideration of optimal dose for clinical efficacy, choice of stabilizer, and behavior of the product during lyophilization. If the dose of vaccine required for clinical efficacy turns out to be higher than that of vaccines now in routine production, loss of titer during lyophilization and stability testing may be relatively more important. The manufacturer must also have in place appropriate facilities and personnel for all internal quality control testing necessary.

Because each of these procedures involves considerable expense, the manufacturer must keep in mind the size of the projected market. Many countries in which vaccines are presently produced do not have populations sufficiently high to make vaccine manufacture cost-effective unless exportation of vaccines is planned.

4.2. Production and Marketing Rights

There are in some cases legal constraints on the ability of a manufacturer to produce a vaccine using a certain vaccine strain and technology. These questions must be clarified before a global policy is developed which may not be realizable due to restrictions on marketing of vaccines.

4.3. Licensing Requirements

Countries manufacturing vaccines have differing requirements. For examples, some countries may produce a vaccine for export which does not have to meet national requirements; others may not. WHO Requirements depend strongly on the role of the national control authority in the country of manufacture to certify the quality, safety, and efficacy of a vaccine. This would presuppose the need for licensing in the country of manufacture if the vaccine were to meet WHO Requirements, unless this responsibility can be shifted to another national control authority. This alternative is not in accord with the current procedure (WHO TRS 771, 1988).

If in the future a recommendation is made to use vaccine of certain characteristics in children under 9 months of age, each producer must independently show in clinical trials that the vaccine produced, at the dose used, gives the desired clinical efficacy. That is to say, each manufacturer's product must be demonstrated to have the desired characteristics.

The current tender for measles vaccine supplied to UNICEF requires that each lot of vaccine be accompanied by a certificate from the national control authority of the manufacturing country stating that the vaccine meets relevant national and WHO Requirements. If a manufacturer produces a vaccine solely for export, it is not clear at present how this requirement will be met. This tender may also need to be modified to reflect the desired titer of an alternative vaccine.

WHO Requirements also specify standards for vaccine stability which must be met. The stability requirements (loss of titer of less than 1 \log_{10} after incubation of the freeze-dried vaccine for 7 days at 37°) may need to be re-examined for use with vaccines that have up to 2 \log_{10} higher titer than the standard vaccines.

Specific stability and potency requirements which may be found necessary for vaccines to be used in children under 9 months of age may be included as an addendum to the UNICEF tender prior to, or in lieu of, changing WHO Requirements. At present, more than 100 million doses of measles vaccine are supplied to UNICEF. This includes vaccines of two types: the Schwarz or Schwarz-like strains produced in chick embryo cells make up the larger proportion of vaccine supplied, and about 5% of the total is E-Z vaccine prepared in HDC. The current

WHO Requirements of potency and stability are the minimum requirements being met at this time by the producers of these two vaccines.

5. TWO DOSE SCHEDULE

Since it became apparent in the 1960's that children were contracting measles before the recommended age of immunization, investigators have been trying alternative strategies to prevent these cases. Some programmes tried giving a first dose at six months and a second at 9 or 12 months (43). This practice virtually ceased when studies revealed that few children who had received a first dose prior to the age of nine months actually returned for a second dose.

As programmes became more successful, cases in older children were prevented, leaving cases occurring before the age of nine months as an increasing proportion of total cases and as an increasing concern. This has rekindled interest in two dose schedules.

There have been two concerns with such schedules, however. The major concern has been programmatic: if the administration of a first dose of a conventional measles vaccines at six months, when it has an efficacy of only some 60%, is associated with lowering of the immunization coverage at 9 months, when it has an efficacy of some 90%, one would expect to observe a sharp increase in measles vaccine "failures" as well as increases in overall cases of measles. It remains the case that relatively few programmes in developing countries have the resources to assure that the majority of children immunized at six months will return again at nine months, limiting the applicability of two-dose schedules.

There has also been an immunological concern. Wilkins (44) and Black (45) both suggested that an early dose of measles vaccine might blunt the immune response to subsequent doses. The issue is two-fold: are the lower antibody titers produced in sero-converters at younger ages sufficient to provide durable immunity, and, if not, are they able to be boosted by a second dose given later in life. The issue of the duration of protection from lower antibody titers requires further study, although observations with the Edmonston-Zagreb vaccine to date are reassuring. Krugman (46), Bass (47) and Shasby (48) all found booster effects with a second dose. Most investigators now seem to feel that the danger of blunting the immune response by providing an early dose of measles vaccine is not a major concern (49).

Where vaccine coverage is high and a significant proportion of cases still occurs before nine months of age, a two dose schedule using conventional vaccines may be attractive. Attention has so far focused on a schedule using standard dose Schwarz vaccine given at both six and nine months. The key question is what percentage of infants must return for the second dose to make the programme equally effective as a single dose programme at nine months?

Indications from mathematical modelling are that this number may be quite small. More precise modelling and related field testing of a two dose schedule are needed.

One assumption in discussing strategies for introduction of an alternative vaccine at six months has been that any change would at this time be for the purpose of controlling measles, not of eradicating it. It may be that at a later date when global coverage is much higher, the issue of a two dose schedule will emerge. The benefits in this circumstance would not be in providing early protection, but in increasing seroconversion rates to the maximum possible.

6. What Studies are Needed Now

6.1. Safety

Alternative measles vaccines appears to be free of any adverse effects which might prevent their widespread use. Use in older children in several countries is reassuring. Clearly any introduction of these vaccines on a wider scale should be accompanied by surveillance, but there is no reason to expect greater numbers of adverse event to be reported than with the presently used vaccines. Adverse events were not increased in eight field trials immunizing younger children reported to a meeting in Washington, D.C., in October, 1988 sponsored jointly by WHO and other international agencies

What is the molecular difference between the E-Z strain and the current measles strains which accounts for its better performance in immunizing the young child? If an explanation for this difference can be identified, it may be possible to design a more immunogenic and safer vaccine.

The existence and possible role of DI particles in the clinical action of the alternative vaccines vaccine needs to be evaluated. Preparations of these vaccine from different manufacturers, which are currently prepared using different techniques, need to be thoroughly studied with respect to molecular biological and clinical parameters. Moreover, this statement holds for any measles virus vaccine: clinical and biological parameters determined for a particular vaccine preparation do not necessarily apply to vaccine preparations of that same strain from another production laboratory and must be independently established for each product and production method.

Studies on the molecular biology of the alternative vaccine strains should be encouraged. This might include the use of monoclonal antibodies and sequencing of DNA complementary to the genomic RNA.

It is not yet clear what is the rate of unusual but serious reactions to alternative vaccines, such as CNS illness (e.g., meningoencephalitis). Is this

rate equivalent to that observed with other currently available strains? These adverse reactions are so uncommon that they frequently escape detection even in large field trials. The current measles vaccine given in the U.S. after 12 months has not been implicated as a cause of subacute sclerosing panencephalitis (SSPE). This complication of wild measles infection has so far only been associated with measles disease at an early age. At least on theoretical grounds, it is important to maintain a careful watch for any evidence that E-Z administration might be implicated with SSPE. However the complete absence of SSPE following conventional measles vaccine is strongly reassuring.

A careful system of post-marketing surveillance might be capable of detecting rare adverse reactions. Such a system may be feasible in industrialized countries, but may be difficult or impossible to establish in developing countries where immunization at early ages is most needed. Case-control studies of rare adverse reactions represent another option for monitoring vaccine safety among very young infants.

In many of the field trials reported so far on adverse effects, the majority of infants had maternal antibodies present at the time of immunization. It is important to know whether adverse events occurred more frequently in infants in whom there were no maternal antibodies at the time of immunization, i.e. in those individuals exposed to the full effect of vaccine virus without any protection from existing circulating maternal antibodies. While there can be confidence as to the overall safety of the alternative vaccines in the under nine month old child, insufficient numbers of infants have been followed to date to be sure that rare events have not taken place. Studies of adverse events should continue as more young children receive the vaccines.

Studies are also needed which follow children who are HIV infected who are immunized at six months with high dose alternative vaccines. Trials of safety and efficacy are needed in areas where HIV prevalence is high such as in parts of Africa. However, there appears to be a balance in favor of the use of such vaccines due to the earlier protection provided against measles in HIV infected children who are likely to suffer drastic consequences from an attack of measles.

6.3. Clinical Efficacy

Two vaccine efficacy studies have been performed, but the number of children involved were small. More studies are clearly needed. Additional studies, particularly in urban areas where outbreaks of measles can be anticipated, would be valuable.

6.4. Duration of Immunity

It is not yet clear how long protection from alternative measles vaccines will last. Will the protection afforded by immunization of very young infants be as durable as that of older infants and children? What if the vaccine induces a very adequate rate of seroconversion at age 6 months but the geometric mean titers fall away over time. Encouraging reports from the Gambia indicate that excellent titers are maintained for at least two years (22). Other preliminary reports are not so reassuring. Studies already under way may be helpful in clarifying this issue when definitive results are published.

6.5. Dose of vaccine

While results so far indicate that E-Z and ATK-C vaccines may both be superior to Schwarz at six months, more work needs to be done in clarifying the optimal dose at this age. For example, there may be an advantage to using even higher doses than those already tested. Additionally, the ability of manufacturers to produce high or very high dose E-Z in a commercial situation is not confirmed. High dose Schwarz vaccine has not been fully investigated, including the possibility of growing the strain in another cell substrate.

It is not yet clear what dose of alternative vaccine needs to be presented in each vial in order for the child in the clinic to receive an immunizing dose. Sub-optimal cold chain conditions are bound to degrade the vaccine to some degree. It will be necessary to estimate the level of "over-kill" needed by manufacturers to ensure an adequate dose of live organisms is present in the syringe at the time of immunization under normal field conditions.

6.6. Standardization of Biological Parameters

Methods of determination of potency of measles vaccine for which clinical efficacy or seroconversion studies are being performed must be standardized and specified. Data should be accumulated comparing sensitivity of different titration systems for the different measles vaccine strains in order to determine the optimal system. Furthermore, thermal stability studies must be done on alternative measles vaccine strains and final formulations (including stabilizers) to insure that the accelerated degradation test specified in the WHO Requirements is applicable to these strains.

If a dose dependence for protection is defined such that a higher dose is needed for use in infants under nine months of age, the maximum titre needed should be included in the UNICEF tender and the WHO Requirements. These requirements should also define how this titre is to be determined and expressed. In the interim, investigators might usefully express measles vaccine potency relative to the International Standard Measles Vaccine (see document WHO/EPI/GEN/88.12 for further details).

A controlled comparability study of different methods of reporting measles positive serology should be performed. Data linking these values to protection against disease would be extremely useful. A necessary first step, however, would be the acceptance of the international standard and international units as a method of reporting serological data.

Further studies need to be done to distinguish infections with wild and vaccine strains. Although an animal model (intracerebral inoculation of marmosets) can potentially be used for this purpose, at present there is no reliable biochemical marker to distinguish between wild and attenuated measles virus.

6.7. Testing in Developing Countries

Now that some of the basic questions of safety and efficacy of the alternative vaccines have been answered, programmes need to look at its gradual introduction. Large urban areas might offer good testing grounds in developing countries for this. Well documented accounts of its introduction at six months are needed, identifying particular problems encountered and any beneficial or other effects.

6.8. Other Alternative Vaccines

While the E-Z vaccine has attracted the most interest from researchers, it is becoming clear, from limited information presented, that other strains are potential candidates for use before nine months of age. These presently include the CAM-70 strain, the AIK-C strain and the Leningrad-16 strain

6.9. Route of Administration

One of the first large trials of E-Z was in Mexico by Sabin et al (44). Here, high dose E-Z was given intra-nasally. It was not clear at that time whether the good results were due to a difference in the vaccine strain or the route of administration. It is now apparent that the strain is superior, but it is less certain whether intra-nasal administration would provide additional benefits to the already superior E-Z strain. Both intra-nasal and conjunctival routes offer theoretically appealing alternatives to injection, as they provide access to the mucous membranes in the same way that wild virus presumably enters the body. In theory at least, this would allow replication of the vaccine virus on the mucous membrane before circulating antibodies (i.e. passive maternal antibodies) could interfere with viral replication. However, recent studies have suggested that the rate of seroconversion is higher with subcutaneous or intramuscular administration than with aerosol delivery (38, 39). Degradation of the vaccine virus by enzymes in the conjunctival fluid is a potential problem for the conjunctival route.

The EPI has a preference for vaccine administration which does not involve skin puncture. Further investigation of these two routes of administration is warranted. Having said this, however, it should be pointed out that the problems to be overcome are considerable. Most field trials of intra-nasal vaccine have been small and unable to cope with the difficulty of regulating the dose of vaccine inhaled. Whether this would be possible on a large scale is questionable. One possible solution might be large droplet aerosols using asthmatic type inhalers.

Interest about the site of administration was provoked by results from hepatitis B vaccine administration (50). When intramuscular injection was attempted into the buttock, results were less effective than if attempted in the arm. Presumably some injections were ending up in the adipose tissue of the buttock and were having less effect than a true intramuscular injection.

Some experience with the intradermal route of administration has suggested that it may be possible to use smaller volumes of vaccine and that there may be a lesser inhibitory effect of maternal antibodies using this route of administration (48). Intradermal injection of vaccine is technically difficult in infants, however.

Alternative routes and sites of administration (i.m. and s.c., intra-nasal and conjunctival) should be considered in an effort to maximize the effect of the alternative vaccines.

6.10. Younger Ages

While most field trials have so far focused on alternative vaccines given at six months, several trials involving children at younger ages have produced promising results. Studies are now indicated to examine the possibility of administration of an alternative vaccine beginning as early as 14 weeks.

6.11. Diagnostic Tests

A test is needed which will readily differentiate between infection from a wild virus and different vaccine strains.

7. CONCLUSION: Considerations for accepting an alternative vaccine.

Before a new vaccine strain is accepted for widespread use, it needs to be assessed in a way that will ensure provision of the best possible vaccine. One system is provided below:

7.1. Does it address a public health problem?

The alternative vaccines potentially solve the dilemma of children developing measles before the age of immunization. This has been a major obstacle in providing effective immunization services to children in many areas.

7.2. Is it safe?

The alternative vaccines have been as safe as the Schwarz vaccine in all reports so far. Completed results from the ongoing field trials will be analyzed for adverse reactions.

7.3. Is it effective?

Two alternative vaccines at medium and high strengths given at six months have been shown to be at least as good as Schwarz given at nine months.

7.4. Is it heat-stable?

Limited data suggest that alternative vaccines are as stable as the Schwarz strain.

7.5. Can it be produced?

While lower dose E-2 can be produced by most manufacturers who have tried, there is still some concern about whether high titre vaccines can be produced at a similar cost to those in current use.

7.6. Is it affordable?

Manufacturers are not yet committing themselves about the change in price that might occur if a switch is made to producing an alternative vaccine. There are some significant differences between production in primary chick embryos and human diploid cells from a cell bank, but it may be that the difference in cost of the final product will be minimal. Many experts outside industry feel that there is very little difference in the method of production, and therefore there should be little or no increase in cost. However, if a high dose is needed, it may be necessary to concentrate vaccine which would increase the cost considerably.

The Global Advisory Group and the EPI Research and Development Group discussed alternative measles vaccine in Abidjan, Cote d'Ivoire, October 1988. Their conclusions and recommendations are included in Appendix 1 and 2.

REFERENCES

1. Expanded Programme on Immunization. Wkly. Epidem. Rec.:1979, 54, 337-339.
2. Hull HF, Williams RJ, Oldfield F. Measles mortality and vaccine efficacy in rural West Africa. Lancet 1983, 1: 972-5
3. Williams RJ, Hull HF. Status of measles in The Gambia, 1981. Rev. Infect. Dis. 1983, 5: 391-4
4. Osagie HF. Delayed mortality and morbidity 12 months after measles in young children in Nigeria. London: Institute of Child Health, University of London, 1986 (MSc Thesis)
5. van de Walle E. Anatomie d'une épidémie de rougeole vue par la lorgnette d'une enquête à passages répétés. In: P. Cantrelle, S. Dormont, P. Farques, J. Goujard, J. Guignard, C. Rumeau-Rouquette (eds). Estimation de la mortalité du jeune enfant (0-5 ans) pour guider les actions de santé dans les pays en développement. Paris: INSERM, 1986: 419-28
6. Aaby P., Bukh J., Lisse I.M., Smits A.J. Measles vaccination and reduction in child mortality: a community study from Guinea-Bissau. J Infect 1984, 8; 13-21.
7. Aaby P., Helm-Petersen N., Stervang B. Relatorio do trabalho preventivo em Bandim I. 1984. mimeo.
8. Bhaskaram P., Reddy V., Raj S., Bhatnager RC. Effect of measles on the nutritional status of preschool children. J. Trop. Med. Hyg., 1984; 87:21-5
9. Loening WEK, Coovadia HM: Age-specific incidence rates of measles in urban, peri-urban, and rural environments: implications for the time of vaccination. Lancet 1983; ii:324-326.
10. Markowitz LE, Bernier RH: Immunization of young infants with Edmonston-Zagreb measles vaccine. Pediatr Infect Dis J 1987; 6:809-812.
11. M. Hirayama, Measles Vaccines Used in Japan, Rev. Infect. Dis. 5, 495-503, 1983
12. S. Makino, Development and Characteristics of Live AIK-C Measles Virus Vaccine: A Brief Report, Rev. Infect. Dis. 5, 504-505, 1983
13. Ikic D, Juzbasic M, Beck M, Hrabar A, Cimbur-Schreiber T. Attenuation and characterization of Edmonston-Zagreb measles virus. Ann Immun Hung 1972; 12:175-181.

14. Gordienko NU, Dorofeev BU. Measles vaccines. In: Guidelines for production of vaccines and sera. Moscow: Medicine, 1978: 220-227.
15. Smorodintsev AA, Boychuk LM, Shikina ES, Meshalova VN, Taros LY, Aminova MG, Revenok ND, Safarov DI. Prevention of measles by the use of live vaccines in the USSR. Arch Ges Virusforsch 1965; 16:284-293.
16. Okuno Y, Ueda S, Kurimura T, Suzuki N, Yamanishi K, Baba K, Takahashi M, Konobe T, Sasada T, Onishi K, Takaku K. Studies on further attenuated live measles vaccine. VII. Development and evaluation of CAM-70 measles virus vaccine. Biken J 1971; 14:253-258.
17. Wegmann A, Glück R, Just M, Mischler R, Paroz P, Germanier R. Comparative study and evaluation of further attenuated, live measles vaccine alone and in combination with mumps and rubella vaccines. Dev Biol Stand 1986; 65:69-74.
18. Sabin AB, Arechiga AF, Fernandez de Castro J et al. Successful immunization of infants with and without maternal antibodies by aerosolized measles vaccine. I. Different results with undiluted human diploid cell and chick embryo fibroblast vaccines. JAMA 1983; 249:2651-2662.
19. Ikić DM. Edmonston-Zagreb strain of measles vaccine: epidemiologic evaluation in Yugoslavia. Rev Infect Dis 1983; 5:558-563.
20. Mann GF, Allison LMC, Copeland JA, Agostini CFM, Zuckerman AJ. A simplified plaque assay system for measles virus. J Biol Stand 1980; 8:219-225.
21. Mirchamsy H, Shafiyi A, Bahrami S, Kamali M, Nazari P, Razavi J, Ahourai P, Fatemi S, Amin-Salehi M. A comparative field trial of five measles vaccines produced in human diploid cell, MRC-5. J Biol Stand 1977; 5:1-18.
22. Whittle HC, Eccles M, Jupp L, Hanlon L, Mann G, O'Neill K, Hanlon P, Marsh V. Effects of dose and strain of vaccine on success of measles vaccination of infants aged 4-5 months. Lancet 1988; i:963-966.
23. Calain P, Roux L. Generation of measles virus defective interfering particles and their presence in a preparation of attenuated live-virus vaccine. J Virol 1988; 62:2859-2866.
24. World Health Organization. Requirements for measles vaccine (live) and measles vaccine (inactivated). Tech Rep Ser 1966, rev 1982, 1988; 329.
25. World Health Organization. Manual of details of tests required on final vaccines used in the WHO Expanded Programme of Immunization. BLG/UNDP/82.1 rev.1

26. Cooper P. The plaque assay of animal viruses. *Adv Virus Res* 1961, 8:319-378.
27. Kenny MF and Schell K. Microassay of measles and mumps virus and antibody in vero cells. *J Biol Standard* 1975; 3:291-306.
28. Lopez Castellanos M, Kado Boll G. Sensibilidad relativa de dos tecnicas para la determinacion de potencia de vacuna antisarampion. XIX Congreso Nacional de Microbiologia 1988.
29. Albrecht P., K.Herrmann, G.R.Burns, Role of Virus Strain in Conventional and Enhanced Measles Plaque Neutralization Test. *Journal of Virol. Methods* 3, 251-260 (1981)
30. Andre FE. Thermodegradation og lyophilized measles vaccines. *Rev Inf Dis* 1983; 5:532-534.
31. Mann GF, Allison IMC, Lloyd JS, Tam P, Zuckerman AJ, Perkins FT. Stability of further-attenuated measles vaccines. *Rev Infect Dis* 1983; 5:482-486.
32. Ueda S, Takahashi M, Minekawa Y, Ogino T, Suzuki N, Yamanishi K, Baba K, Okuno Y, Konobe T, Sasada T, Takaku K, Kurose T. Studies on further attenuated live measles vaccine. II. Correlation between the titer of the vaccine, the antibody response, and clinical reactions. *Biken J* 1970; 13:117-120.
33. Isomura S, Morishima T, Nishikawa K, Hanada N, Rahman M, Terashima M, Kido S, Ueda S, Takahashi M. A long-term follow-up study on the efficacy of further attenuated live measles vaccine, Biken CAM vaccine. *Biken J* 1986, 29:19-26.
34. Peradze TV, Smorodintsev AA. Epidemiology and specific prophylaxis of measles. *Rev Infect Dis* 1983, 5:487-490.
35. Beck M, Smerdel S, Dedic I, Delimar N, Rajninger-Miholic M, Juzbasic M, Manhalter T, Vlatkovic R, Borcic B, Mihajic Z. Immune response to Edmonston-Zagreb measles virus strain in monovalent and combined MMR vaccine. *Dev Biol Stand* 1986; 65:95-100.
36. Sabin AB, Arechiga AF, Fernandez de Castro J, Albrecht P, Sever JL, Shekarchi I. Successful immunization of infants with and without maternal antibody by aerosolized measles vaccine. II. Vaccine comparisons and evidence for multiple antibody response. *JAMA* 1984; 251:2363-2371.
37. Whittle HC, Rowland MCM, Mann GF, et al.: Immunization of 4-6 month-old Gambian infants with Edmonston-Zagreb measles vaccine. *Lancet* 1984; ii:834-837.

38. de Castro JF, Gomez JLV, Ortega JLD, et al.: Diploid cell measles vaccine. JAMA 1986; 256:714.
39. Khanum S, Uddin N, Garelick H, et al.: Comparison of Edmonston-Zagreb and Schwarz strains of measles vaccine given by aerosol or subcutaneous injection. Lancet 1987; i:150-153.
40. Aaby P, et al, Trial of high dose Edmonston-Zagreb measles vaccine in Guinea-Bissau: protective efficacy. Lancet 1988;i :809-811.
41. Beck M, Smerdel S: Clinical studies of the Edmonston-Zagreb measles vaccine in Yugoslavia. Presented at Workshop for Use of Measles vaccine In Infants under 9 Months of Age. Centers for Disease Control, Atlanta,GA, November 21 to 22, 1985.
42. Whittle HC: Clinical trials in the Gambia. Presented at the Workshop for Use of Measles Vaccine in Infants under 9 Months of Age. Centers for Disease Control, Atlanta, GA, November 21 to 22, 1985.
43. Wood PB., Schendra K.S., Branken P.M. and Houser N.E.: Measles vaccination in Zaire - when and how? Trans. R. Soc. Med. Hyg. 74: 381, 1980.
44. Wilkins J., Wehrle P.F. Additional evidence against measles vaccine administration to infants less than 12 months of age: altered immune response following active/passive immunization. J. Pediatr. 94: 865, 1979.
45. Black F.L., Berman L.L., Libel M. et al. Inadequate immunity to measles in children vaccinated at an early age: effect of revaccination. Bull. WHO. 62 : 315, 1984.
46. Krugman S., Giles P.J., Friedman H. and Stone S.: Studies in immunity to measles. J. Pediatr. 66:471, 1965.
47. Bass J.W., Halstead S.B., Fisher G.W., Podgore J.K., et al. Booster vaccinations with further live attenuated measles vaccine. JAMA 235: 31, 1976.
48. Shasby D.M., Shope T.C., Downs H. et al. Epidemic measles in a highly vaccinated population. N. Eng. J. Med. 296:585, 1977.
49. Stetler H.C., Orenstein W.A., Bernier R.H. et al. Impact of revaccinating children who initially received measles vaccine before 10 months of age. Pediatrics, 77:471, 1986.
50. CDC. Suboptimal response to hepatitis B vaccine given by injection into the buttock. MMWR 1985; 34: 105-13.

APPENDIX 1.
Recommendations of the Global Advisory Group,
Abidjan, October 1988.

A number of investigators are currently completing large studies comparing different strains and potencies of measles vaccines administered to infants prior to the age of nine months. Preliminary data from these studies were reviewed and discussed by the Research and Development Group at its October 1988 meeting and by the Global Advisory Group.

The results obtained to date from this group of studies were judged very encouraging. They suggest that one or more vaccines will be identified which will be suitable for routine use in infants at high risk of exposure to measles. However, because the studies are still incomplete, several key questions cannot be answered confidently at this time.

It was concluded that these data do not yet warrant a recommendation to administer routinely any higher than standard potency measles vaccine to infants below nine months. The higher than standard potency Edmonston-Zagreb vaccine presently being supplied to some countries is included in this restriction.

Countries should not change from using the existing schedules and strains of measles vaccines. When the data on safety, immunogenicity, and efficacy become more complete, as is expected over the next one to two years, such data will be re-evaluated to determine if a change in the present recommendations can be supported.

Research should be pursued on the impact of higher than standard potency measles vaccines in some selected urban areas of known high risk. In addition, clinical and laboratory studies of different strains at varying doses should be initiated.

Achieving high coverage with existing vaccines remains the first priority in all countries.

The age of immunization should be based on the current epidemiology of measles. Where disease occurs principally among older infants and young children, routine immunization should begin as soon as possible after the age of nine months.

In special populations at particularly high risk of measles morbidity and mortality before nine months (such as hospitalized children, those affected by disasters or in refugee camps) immunization should begin as soon as possible after the age of six months. All children receiving a measles vaccine prior to the age of nine months should be reimmunized as soon as possible after the age of nine months.

APPENDIX 2.

Recommendations of the EPI Research and Development Group Meeting,
Abidjan, October 1988.

The Research and Development Group reviewed with satisfaction progress on the implementation of studies to measure the effectiveness of alternative strains of measles vaccine administered in high titer at six months of age. While the data available are still preliminary, they strongly suggest that high potency measles vaccines are effective in providing high rates of seroconversion and measles protection. Continuing data analyses and follow up studies will be required to provide sufficient data to formulate new policy recommendations. The potential of this development to significantly enhance global capability to prevent childhood morbidity and mortality is major. Based on discussions during the Research and Development Meeting and in a follow up subgroup meeting chaired by Prof. Kostrzewski, the group makes the following recommendations :

1. Continue to Use Current Measles Vaccines

1.1 WHO should continue to recommend immunization with currently available vaccines at nine months or as soon as possible thereafter. The challenge for all programmes is to achieve high coverage with available vaccines.

2. Continue Studies on Alternative Measles Vaccines

2.1 Ongoing studies of alternative measles vaccines administered at or before six months should be completed. Special attention should be paid to follow the duration of vaccine induced antibodies and of vaccine failure rates in children vaccinated at or before 6 months of age. As much as possible methodologies should be standardized to permit comparison of results among studies. EPI Geneva should provide a standardized format to investigators, recommending standard analyses that WHO would like to have.

2.2 Promote additional studies in different geographical regions on the use of alternative measles vaccines. Of particular priority are :

2.2.1. studies which compare sero-responses after immunization at six months with other vaccine strains (at various titers and in relation to pre-existing levels of maternal antibody) with the Schwarz and E-Z strains.

2.2.2. studies which compare sero-response after immunization between the ages of 14 and 24 weeks with different measles vaccines (at various titers and in relation to pre-existing levels of maternal antibody).

2.3. Follow-up discussions on the status of current studies and on information needed for policy alteration should be given high priority at the EPI Research and Development Meetings in March 1989.

3. Additional Studies of High Titer Vaccines

3.1 Studies to evaluate additional high titer vaccine strains at or before 6 months should be encouraged.

4. Research in Urban Areas

In urban areas where measles transmission rates are high under 12 months of age and control has not been achieved with standard vaccine administered at 9 months of age, studies to measure the effectiveness of high potency vaccines at 6 months should be undertaken. Studies need to be limited to areas that have ongoing systems of surveillance to measure changes in morbidity and to areas for which sufficient supplies of high potency vaccine are assured.

5. Two-Dose Measles Vaccine Schedule

5.1 Children admitted to hospital facilities are at high risk for both morbidity and mortality. All at risk age groups 6 months to five years should be vaccinated with measles vaccine on admission. Children vaccinated under nine months should be reimmunized at 9 months.

5.2 Children living in situations where measles below 9 months of age constitutes a major health risk e.g. urban slums, disaster affected populations, refugee camps, should be vaccinated at 6 months of age. All such children should be reimmunized at 9 months of age.

5.3. Promote further operational research using standard vaccines from the age of six months to assess the impact of two dose immunization strategies in areas where measles transmission rates remain high despite immunization at the age of nine months. Studies should be limited to those areas which have surveillance systems sufficiently developed to be able to measure changes in measles mortality.

6. Vaccine Manufacturers

6.1 EPI should contact vaccine manufactures to evaluate the possibility of increasing the titer of measles vaccines currently being provided to UNICEF and EPI.

6.2 EPI needs to explore with manufactures options for production of high potency measles vaccine strains.

7. Other Areas of Research

7.1. Studies of the safety of high titer vaccines in older non immune children.

7.2. A study should be initiated to further evaluate the feasibility and effectiveness of alternative routes of administration of high dose vaccines, particularly intranasally, conjunctivally and intradermally, and at different ages.

7.3. Studies should be performed to determine the reason why Edmonston-Zagreb vaccine results in higher response rates than Schwarz vaccine in infants with maternal antibodies.

7.4. Studies should be performed to determine the safety and immunogenicity of high dose vaccines in HIV-infected children.

7.5. Basic research to characterize the molecular biological characteristics of vaccine strains which appear to be more successful in inducing immunity in the face of maternal antibodies.

* * *