

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES

No. 181

**IMMUNOLOGICAL
AND HAEMATOLOGICAL SURVEYS**

Report of a Study Group

	Page
1. Introduction	3
2. Studies on the blood of man and animals in which survey methods are indicated	5
3. Uses and limitations of serological surveys	16
4. Methods of sampling the population	19
5. Technical considerations regarding the methods of collection, shipment and treatment of blood for multi-purpose examination	27
6. Suggested list of items of information to be recorded in surveys	30
7. Criteria for release of specimens	32
8. Standardization of tests	32
9. Recording and reporting of results	33
10. Recommendations for the development of multi-purpose blood surveys and of an international collection of sera	33

WORLD HEALTH ORGANIZATION

PALAIS DES NATIONS

GENEVA

1959

**STUDY GROUP
ON IMMUNOLOGICAL AND HAEMATOLOGICAL SURVEYS**

Geneva, 15-19 December 1958

*Members : **

Dr J. B. Brooksby, Deputy Director, Research Institute (Animal Virus Diseases), Pirbright, Surrey, England

Dr J. Casals, Staff Member, The Rockefeller Foundation Virus Laboratories, New York, N.Y., USA

Dr D. Horstmann, Associate Professor of Preventive Medicine and Paediatrics, Yale University School of Medicine, New Haven, Conn., USA (*Rapporteur*)

Professor K. Raska, Institute of Epidemiology and Microbiology, Prague, Czechoslovakia (*Vice-Chairman*)

Dr F. Yates, Rothamsted Experimental Station, Harpenden, Herts., England (*Chairman*)

Representative of the Food and Agriculture Organization :

Dr E. Eichhorn, Chief, Animal Health Branch, FAO

Secretariat :

Dr G. Arroyave, Chief, Clinical Biochemistry Division, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala, C.A. (*Consultant*)

Dr J. H. P. Jonxis, Professor of Paediatrics, State University, Groningen, Netherlands (*Consultant*)

Dr A. M.-M. Payne, Chief Medical Officer, Endemo-Epidemic Diseases, Division of Communicable Diseases, WHO (*Joint Secretary*)

Dr B. Pirc, Chief Epidemiologist, Consolidation of Health Statistics, Division of Health Statistics, WHO (*Joint Secretary*)

** Invited but unable to attend :*

Professor E. N. Levkovich, Chief, Encephalitis Laboratory, Institute of Virology, USSR Academy of Medical Sciences, Moscow, USSR

This report was originally issued in mimeographed form as document WHO/Imm.Surv./4.

IMMUNOLOGICAL AND HAEMATOLOGICAL SURVEYS

Report of a Study Group

The Study Group on Immunological and Haematological Surveys met in Geneva from 15 to 19 December 1958. Dr F. Yates was elected Chairman; Professor K. Raska, Vice-Chairman; and Dr D. Horstmann, Rapporteur.

1. INTRODUCTION

The development and maintenance of sound public health programmes depend on knowledge of the prevalence and distribution of disease and the changes which occur in these over the years. In most countries an attempt is made to obtain this information by recourse to morbidity and mortality data. It is however recognized that the value of such data varies widely, both from country to country and from disease to disease. In many areas and for many diseases, owing to diagnostic difficulties and other factors, the data have little or no validity.

Another method of gathering this information is by means of surveys in which, by appropriate serological tests or by skin sensitivity tests, evidence of past or recent activity of an infection can be obtained, together with an estimate of its prevalence. This approach gives information which goes beyond that obtained by other means, for it can serve in a precise way as an epidemiological history of what has happened in the population over the past years. A serological survey provides information regarding the prevalence of infection but does not give direct evidence of the prevalence of disease; it has nevertheless a special value since, in conjunction with other epidemiological information, it enables an estimate to be made of the public health importance of many infections, as well as an appreciation of the potential importance of infections which may be clinically inapparent at the time but which may still be transmissible or may become clinically apparent in the future, sometimes in epidemic form, under the influence of changing epidemiological circumstances. Furthermore, information will be obtained regarding the presence of infections which may be responsible for part of the mass of undiagnosed illnesses in the country, including those in which the clinical diagnosis is of doubtful validity. Measures can then be taken to make a more specific diagnosis of these cases. In addition, information regarding the immunity status of the population is of great value in planning immunization programmes

against some diseases, since the section of the population which is susceptible and which requires immunization can be identified.

A study of the curves of antibody distribution by age throws useful light on the rate of spread of infection in the past, as well as the age at which infections are acquired, and makes it possible to examine the impact which ways of living have on the epidemiology of the infection in different areas and in different population groups—i.e., age specific rates under conditions of poverty or affluence, urban or rural living, or according to occupational groups in a given population.

Early examples of serum surveys were those listing the results of Wassermann tests in samples from normal populations representing Negro and white people in various areas, usually cities. In the 1930's the first important geographical surveys of the prevalence of antibodies against yellow fever virus, carried out for the most part in Brazil and Africa by the International Health Division of the Rockefeller Foundation, demonstrated the existence of jungle or sylvatic yellow fever. Later, in the 1940's, serum surveys were used for mapping the geographical distribution of the arthropod-borne virus encephalitides, and soon afterwards they were applied to poliomyelitis. More recently, some investigators have examined the sera collected using batteries of tests, thus obtaining information regarding several diseases at little extra cost, since many different antibodies can be demonstrated in a single 10-ml sample of serum. However, experience of such multi-purpose surveys is limited and the statistical and epidemiological conditions which must be met in order to ensure their validity are not widely known.

The pattern of communicable diseases through the world is constantly changing, owing to natural variation of infectious agents, their ecology, the animal reservoirs, natural and man-made changes in the environment, and changes in man and his behaviour. Also it may be safely assumed that in the future the causative agents of many diseases which are at present unknown will be discovered. If samples of the sera collected in these surveys are stored in such a way as to preserve antibodies, it will be possible to examine them in the future and so to determine the past history of infections as yet unknown and to follow more clearly the changing pattern of communicable diseases all over the world. The potential value of such collections to the epidemiologist needs no emphasis. For example, if they had been made before and after the 1918-19 pandemic of influenza and stored for examination a decade later, when the influenza virus was first discovered, we should know much more than we do now about one of the great disasters in the history of the human race.

Since animals are the reservoirs of many communicable diseases of man and play a major role in the epidemiology of many human infections, it would clearly be of great value to apply serological epidemiology to the study of these infections in their animal hosts, as well as in man.

A serological survey is based on the collection of blood from samples of populations, selected in some acceptable manner. But blood contains many substances other than antibodies, the examination of which is of public health importance and of scientific interest to experts in a number of different disciplines. Haematological and biochemical investigations are of great importance in the study of certain chronic diseases, of over- and under-nutrition, and of hereditary abnormalities. It is logical, therefore, to consider whether the blood samples can be collected in such a way as to make it possible to carry out examinations related to these conditions as well, thus increasing the amount of useful information obtained from the survey at relatively little extra cost. It is pointed out that, especially in less well developed countries and in relatively inaccessible areas, the cost of a survey consists mainly of expenditure on the survey team, its equipment and its transport to the area under study. Broadening the scope of surveys in this way to include haematological and biochemical tests implies little or no addition to the cost of this major item, the only extra outlay needed being for some equipment and laboratory examinations. Furthermore, taking blood from populations, especially from the most important younger age-groups, is not easy and when it is done the maximum use should be made of the material collected.

The Study Group has given close attention to all these various aspects of multi-purpose surveys, based on the collection of blood from samples of human and animal populations, and in the paragraphs which follow will be found the conclusions of the Group, and the considerations on which they are based, regarding the feasibility of the broad approach outlined above, together with proposals for its application in practice.

Although skin tests for communicable diseases are not discussed in this report, the statistical and epidemiological requirements in surveys designed for serological or skin tests are very similar, if not identical, and there is much to be said for combining them when that is feasible.

2. STUDIES ON THE BLOOD OF MAN AND ANIMALS IN WHICH SURVEY METHODS ARE INDICATED

Subjects which lend themselves to study by serological and haematological surveys fall into several categories, depending largely on the availability of suitable tests. Four major categories may be considered :

- (1) Immunological studies on the past and present prevalence of infections in man and animals ;
- (2) Genetic studies, such as those on blood groups and abnormal haemoglobins ;
- (3) Studies of anaemias of various types :

(4) Biochemical studies, especially those related to nutrition, such as the determination of plasma proteins and cholesterol.

2.1 General administrative considerations

General problems in connexion with surveys covering such a wide range of fields are numerous and complex. In developing a world-wide programme of this kind, it is important to designate world centres for each group of subjects or diseases, plus a network of co-operating regional laboratories in different countries, comparable to the WHO influenza network. The policy of WHO is not to set up new laboratories, but to utilize existing laboratories in various parts of the world—laboratories which are already staffed by interested and competent persons, who can undertake co-ordinated research in their particular fields. Before such work is undertaken, however, it would seem advisable to collect the existing information on the various subjects—particularly some of the now more accessible data from the USSR and other countries—abstract this and circulate it among those interested. Once an organization of co-operating laboratories is set up, means should be devised for the regular dissemination and exchange of new information, including regular reports of activities of the central laboratory and of the collaborating laboratories, etc., as well as the publication by WHO of new information and results.

No doubt large stocks of human and animal serum samples exist in store in many laboratories. Inquiries should be made concerning the existence of such stocks and steps taken to preserve, catalogue and utilize them if suitable for the purposes outlined in this report.

2.2 Objectives

Two types of survey are envisaged :

(1) Specific surveys, initiated to obtain answers to specific problems, on to which may be grafted

(2) Multi-purpose surveys, from which information can be obtained concerning two or more of the four main fields of study listed above. Thus it is visualized that a team undertaking a survey for a specific purpose in a given geographical area would be able to include collections of suitable specimens for other determinations, depending on the area, the local problems and local interests, as well as the interests of regional laboratories in the vicinity. This type of co-operation requires specific foreknowledge on the part of the collecting team of the needs of other groups.

It is probable that in some instances the two types of collection will be compatible, while in other situations they may not.

An important aspect of the multi-purpose survey is the proper storage of serum for future reference, conceivably ten to twenty years hence.

Such a collection would have great value in the study of the spread of a newly recognized disease and in the utilization of new tests which may be devised for previously known diseases (e.g., hepatitis) or which may have a bearing on biochemical and haematological problems.

2.3 Suggestions for surveys

2.3.1 *Immunological surveys of human sera*

Collections of sera for these surveys will often be made on the basis of some immediate problem in a given local area. Indeed the existence of such a problem may provide the main argument for persuading populations to co-operate. However, the ultimate goal includes the mapping of the world-wide distribution of various infectious agents.

Most virus diseases of particular interest can be grouped under headings of arthropod-borne (arbor), respiratory and enteroviruses.

2.3.1.1 *Arbor viruses*

A considerable amount of exploratory work has already been done by several agencies and individuals in different parts of the world. These investigations should be continued and expanded ; in addition, the following areas, about which little is known, are considered of particular interest and recommended for investigation :

(1) The Mediterranean basin and some adjacent areas, including Romania, Bulgaria, Turkey, Greece, Albania, Yugoslavia, Italy, the Iberian Peninsular and North Africa west of Egypt. Extensive surveys have already been done in Egypt and, to some extent, in Israel, but little information is available about the rest of the area. This region is considered of interest owing to the proximity of foci of infection such as those caused by the West Nile and Sindbis viruses in Egypt ; in addition, it is known that at least three arthropod-borne virus diseases—namely, the two sandfly fevers and dengue—have existed in the area at one time or another.

(2) East Africa (Mozambique and north to Somaliland). This area is considered important as a possible link between Equatorial Africa and Asia, particularly the Arabian Peninsula and India.

(3) Many of the countries of Western Europe. The presence of the Russian spring-summer tick-borne group of viruses or of antibodies against them in countries from Sweden to Yugoslavia makes it particularly pressing to survey the areas from the Baltic to the Adriatic seas and westward.

(4) South America. The Caribbean area, certain parts of Brazil and Colombia have already been and still are the subject of study. The rest of South America is by and large unexplored. It is thought that by surveying certain parts of this area, information may be obtained on, among

other questions, possible pathways of progression and dispersion of viruses by migrating birds.

Animal reservoirs, particularly birds, should be included in certain surveys, since migratory birds may form an important link in the epidemiological chain. Where yellow fever or dengue viruses are known to exist, collection of bloods from monkeys of the area is indicated. The possibility that hibernating animals may serve as over-wintering hosts for certain of the arbor viruses also deserves consideration.

For large-scale surveys, group testing (for groups A, B and C) is most effectively carried out by means of the haemagglutination-inhibition (HAI) test, which requires a minimal amount of serum. 0.5 ml is sufficient to carry out tests against 10 antigens. If a large number of positive reactors with high titres is found for a given virus or viruses, further studies in the area are indicated. These include neutralization tests to aid in designating the specific agent responsible, and, most important, efforts to isolate the virus or viruses from the area. The latter is the only certain way of establishing the activity of a given virus in a given area.

2.3.1.2 *Respiratory viruses*

Influenza studies are at present well taken care of and should be continued by the World Influenza Centre, the International Influenza Centre for the Americas and co-operating national laboratories. It would seem logical to extend the work of the influenza laboratories to include the myxoviruses generally (and other respiratory viruses) as their number and importance increase. At present most of these viruses are so new that investigations are at the pilot study stage, in which isolation and identification of agents and correlation of serological responses are being actively studied. It is probably too early to plan extensive serological surveys in connexion with these agents, although collections of sera made now may be examined in the future when appropriate tests are developed. However, the testing for group CF antibodies against adenoviruses might be suggested as a starting point.

Surveys for the psittacosis-ornithosis group are also indicated under certain circumstances.

2.3.1.3 *Poliovirus*

The serological survey approach has been used extensively in studying poliomyelitis. Enough data have been collected to indicate that the antibody patterns of a given community correlate well with the reported incidence of the paralytic disease there; where reporting is reasonably accurate, therefore, the immunity of a population may be estimated on the basis of the history of recorded attack rates.

Thus, as noted in the second report of the WHO Expert Committee on Poliomyelitis,¹ at the present time serological surveys are indicated only in certain special circumstances, as follows :

- (1) Areas where adequate statistics on disease are not available ;
- (2) As an aid in assessing the immunological status of a population in order to determine whether, when, and in what age-groups, vaccination programmes should be carried out. (This is of particular importance to local health authorities.)
- (3) On islands and in other isolated communities where the immunity status is unpredictable ;
- (4) In connexion with epidemics showing unusual features difficult to interpret.

In addition, examinations of animal and avian sera for poliovirus antibodies may deserve re-exploration.

2.3.1.4 *Enteroviruses other than poliovirus*

This field has been little studied owing to its newness and to the fact that so many agents have been discovered over such a short period of time. However, the evidence to date suggests that there is little antigenic crossing between various ECHO viruses, which makes their study relatively easy. It would probably be of most value to begin with the ECHO types which have been shown to produce epidemics of disease.

Serological surveys of Coxsackie virus infections have been limited by the size and complexity of the group and by problems related to antibody tests—e.g., the marked cross-reactions with certain members of the group by the CF test. With group B viruses (and certain group A viruses which grow satisfactorily in tissue culture) neutralization tests could be used, for instance, to survey populations in the event of unusual epidemic conditions.

2.3.1.5 *Other diseases*

Measles. The newly developed complement-fixing and neutralizing-antibody tests against measles virus have made the study of the epidemiology of measles far more exact than has ever been the case before. However, owing to the specific nature of the clinical picture and to the fact that by far the majority of cases are apparent, morbidity data are more reliable than in the case of most infections ; the serological approach is probably of more importance in ascertaining the susceptibility of individuals and whether or not they need protection, etc., than in determining the immune

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1958, **145**, 19-20

pattern of a community. Nevertheless, the correlation of antibody patterns with the history of measles in remote areas such as Greenland, and in isolated communities such as the Pacific islands, would be a matter of considerable public health interest, particularly as vaccination against this disease may become possible in the not too distant future.

Also in connexion with measles antibody surveys, attention should be given to the significance of the immunological relationship between measles and distemper viruses. A survey of dog sera early and late in the course of a measles outbreak might be an interesting project.

Hepatitis and infectious mononucleosis. Although at present no suitable tests are available for a serological survey of these diseases, it is important wherever possible to collect and store sera, until such time as satisfactory study methods have been developed.

Rickettsial infections. On a world-wide basis, studies on Q fever antibody in man and animals would contribute appreciably to epidemiological knowledge of this disease, especially from the point of view of natural reservoirs and progression of the disease geographically. For other rickettsial diseases such as typhus fever (epidemic and endemic), scrub typhus, rickettsialpox and the various spotted fevers (e.g., Rocky Mountain, Brazilian, Colombian, boutonneuse, Kenya, Indian, South African) surveys in geographical areas having characteristics and fauna similar to those of known infected areas should provide important epidemiological information with respect to these diseases.

Rheumatic fever. Streptococcal infection, in relation to rheumatic fever, is an important field. The exploratory use of the antistreptolysin-O (ASLO) test under varying conditions may be considered.

Rheumatoid arthritis. In this disease the sensitized sheep cell test and the new modifications using latex particle agglutination offer exploratory possibilities for epidemiological studies of subclinical as well as clinical cases.

Bacterial infections. Many bacterial infections lend themselves to serological study for epidemiological purposes. It is probable that improved techniques will be forthcoming with respect to the already well studied infections such as those caused by streptococci, staphylococci, diphtheria, typhoid and other salmonellae, so that better epidemiological information will be obtained than is now available. Much more information is needed, and will continue to be needed, with respect to global prevalence and spread of brucellosis and listeriosis in man and animals. Serological studies with respect to plague, particularly the animal reservoirs of this disease, would be of value in locally affected areas.

Spirochaetal infections. Although extensive surveys for yaws and syphilis have been carried out there are still numerous areas where further

work is indicated. Other treponematoses, relapsing fever and leptospirosis should also be investigated by serological survey techniques.

Parasitic infections. Toxoplasma antibody surveys have a high priority in both human and animal populations. Such surveys will no doubt provide very useful information for clarifying the at present obscure epidemiology of the disease. While serological techniques for toxoplasmosis as well as other protozoal and helminthic and mycotic diseases require much improvement, advances in such techniques in future years will no doubt contribute greatly to the value of the collections of serum specimens discussed in this report.

2.3.2 *Immunological surveys of animal sera*

It is very desirable that a representative sample of sera from domestic animals (cattle, sheep or goats, swine, horses, dogs and fowls) should be included as a part of any survey of human sera. The difficulty in organizing such surveys of animal populations might be overcome by linking the project to surveys already in progress—such as, for example, brucellosis studies—or by collecting sera at veterinary schools, veterinary clinics, or abattoirs.

The collection of sera from wild animals and birds is known to be of great importance in the study of some infections. Further exploration of this field is desirable.

Animals closely associated with humans, as well as those found in uninhabited areas, should be considered for inclusion in the sample, depending upon local conditions. Here collection of samples by groups dealing with zoological surveys might avoid the difficulty of initiating new independent operations. Such use of existing surveys might result in the selection of unsuitable samples but would be preferable to complete omission of the material.

Wherever a new series of serum collections is begun by individual groups for a specific aim, the possibility of extension to satisfy future requirements in WHO co-ordinated studies should be considered. WHO should explore the present programmes of other organizations with a view to taking advantage of their activities in obtaining the desired serum specimens of domestic and wild species.

The following list indicates the species of animals which should be considered for inclusion, as well as some examples of the diseases or pathogens of which they are important hosts.

Dogs :	Rickettsial diseases, leptospirosis, rabies, leishmaniasis, toxoplasmosis
Cattle :	Brucellosis, leptospirosis, Q fever, psittacosis-lymphogranuloma group

Sheep and goats :	Brucellosis, tularaemia, Q fever, tick-borne encephalitis, listeriosis, psittacosis-lymphogranuloma group
Swine :	Brucellosis, listeriosis, toxoplasmosis, influenza, Sendai-virus diseases, arborviruses
Horses :	Arborviruses, influenza
Domestic fowls :	Salmonellae, listeriosis, psittacosis-ornithosis, arborviruses, Q fever, myxoviruses
Wild birds :	Psittacosis, Q fever, arborviruses
Rodents :	Plague, murine and scrub typhus, arborviruses, Q fever, tularaemia, leptospirosis, toxoplasmosis
Primates :	Jungle yellow fever and other arborvirus diseases
Bats :	Rabies, arbor and other viruses

Wild analogues of the domestic species (e.g., wild hogs and dingoes) can be similarly examined.

These general considerations should serve to stimulate further studies at natural foci of diseases, the importance of which has already been demonstrated in relation to various arborviruses, Q fever and influenza. Such special collections of sera should be catalogued along with the human sera.

2.3.3 *Blood groups*

Surveys for blood groups can be considered from the point of view of their possible relationship to susceptibility to disease or in terms of their anthropological significance. The blood group is a useful genetic marker and will provide valuable data for comparative genetics, especially if followed in successive generations.

At present, surveys should concentrate on furthering knowledge of the world-wide distribution of blood groups. Later, possible relationships to disease can be explored. Ethnic factors are of primary importance in blood-group surveys. Such surveys have their maximum value if carried out in homogeneous groups of a given population rather than if attempts are made to cover an entire country. Specimens collected in the course of multi-purpose surveys should be accompanied by the appropriate ethnic and familial data on the individuals sampled.

2.3.4 *Haemoglobinopathias*

Abnormal haemoglobins are important to human health for several reasons :

(1) In those populations in which the percentage of carriers of one or more of these hereditary abnormalities is high (as in parts of Africa, Asia and America), about 1% of the children born are homozygous or double heterozygous, for instance, with respect to the sickle cell and the Cooley gene. With a few exceptions these children will die in their first years of life.

(2) The heterozygous carriers of these hereditary abnormalities are less severely affected, but often they have a more or less severe anaemia which should be distinguished from anaemias caused by iron deficiencies, intestinal parasites and malaria.

(3) Carriers of the sickle cell and Cooley genes may enjoy a certain protection against malaria. It is likely that the presence of the genes for some other abnormal haemoglobins is, under certain conditions (e.g., iron deficiency), an advantage to their carriers.

In many parts of the world individuals heterozygous with respect to one of these haemoglobinopathias are common. These areas include North and Central Africa, Italy, Greece, the Eastern Mediterranean countries, India, Pakistan, Burma, Thailand, Indonesia, and those parts of the world in which there are immigrants from these countries.

When blood surveys are made in areas of special interest in terms of abnormal haemoglobins, the number of carriers of haemoglobinopathias should be determined.

2.3.5 *Anaemias*

The Study Group noted the observations of the WHO Study Group on Iron Deficiency Anaemia that iron deficiency anaemia appears in many areas as a major public health problem. At present there is inadequate knowledge of the world-wide prevalence of this condition. It is recommended that at least determinations of haemoglobin and haematocrit should be carried out in the proposed surveys. The determination of the red blood cell count is desirable but at present the methods available suffer from considerable inaccuracy and are not considered of practical use for large-scale survey work. In surveys of haemoglobin and haematocrit values, particular attention should be given to infants, children and pregnant women.

The necessary technical considerations are fully discussed in the Report of the Study Group on Iron Deficiency Anaemia.¹ It is believed that information on total haemoglobin levels could be important also in connexion with the determinations of abnormal haemoglobins proposed in section 2.3.4.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1959, **182**

2.3.6 *Biochemical nutritional studies*

The Study Group recognizes that at the present time nutritional problems in different parts of the world vary widely. In certain under-developed areas, under-nutrition is still the major problem, while in other, more highly developed, countries, over-nutrition has become an important health challenge. The biochemical investigations recommended for the surveys contemplated attempt to cover both types of problem.

2.3.6.1 *Plasma proteins*

A better knowledge of serum proteins is urgently needed for an understanding of the metabolic alterations occurring in such conditions as protein malnutrition and diseases in which protein breakdown is accelerated or in which there is protein loss. In order to contribute basic data about this question it would be desirable to determine the total plasma protein and its fractions in the population surveyed. The findings might throw light on new aspects of known specific pathological disorders. They might also provide basic biochemical information for future studies—for example, in investigations of hereditary peculiarities in the formation of certain plasma protein fractions.

2.3.6.2 *Plasma vitamin studies—vitamin A*

The scattered studies carried out up to the present indicate that vitamin A deficiency is one of the most serious forms of vitamin lack undermining the health of population groups in certain parts of the world. The Joint FAO/WHO Expert Committee on Nutrition, in its Fifth Report, stressed the seriousness of the problem as follows :

In parts of Ceylon, India, Indonesia, Malaya, and the Philippines keratomalacia is reported to be a frequent cause of blindness. Deficiency of vitamin A in the diet is common in parts of South-East Asia, Latin America, and Africa. There is evidence that vitamin A deficiency leads to pathological states other than severe disease of the eye in these regions.¹

In view of this consideration it seems advisable to investigate vitamin A deficiency more thoroughly among many population groups. It has been well established that, under ordinary circumstances and within a certain range, the levels of vitamin A or vitamin A plus carotene in the plasma or serum are related to intake and to liver storage. Plasma values of 15-20 mg/100 ml are presumptive evidence of a deficiency that may or may not be clinically apparent.

It is therefore recommended that plasma vitamin A and carotene levels be determined in the blood specimens collected during certain of

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1958, 149, 33

the proposed immunological surveys. The results of these determinations could reveal deficiencies as yet unknown in some areas of the world and contribute to a better understanding of the epidemiology of vitamin A deficiency. Since there is already evidence that pathological conditions such as certain infectious diseases may also lead to low plasma vitamin A values, the data thus obtained may possibly lend themselves to further interpretation in this respect.

2.3.6.3 *Other vitamins*

The Study Group recognized the importance of more-complete investigations of other vitamins of known or suggested public health significance, but unfortunately at the present time practical limitations prevent their study within the scope of the types of survey discussed in this report.

2.3.6.4 *Plasma lipids*

Serum cholesterol levels are known to vary among different groups of populations in the world. These differences have been attributed to various causes among which environmental factors are considered the most important.

A specific relationship between serum cholesterol levels and the quantity and quality of dietary fat has been accepted by many investigators, but the role played by other dietary factors, such as total caloric intake or proteins, cannot be defined on the basis of present knowledge.

The Study Group on Atherosclerosis and Ischaemic Heart Disease¹ considered that estimates of serum cholesterol and lipoprotein fractions, among other substances in the blood, may come to be used widely in community studies of atherosclerosis. It is felt that the determination of these fractions in the blood plasma collected in the proposed immunological surveys would contribute to the critical evaluation of the usefulness of these tests as epidemiological tools.

2.3.6.5 *Other biochemical measurements*

There is suggestive evidence that individuals living under conditions of dietary protein restriction have lower blood plasma levels of some non-protein nitrogen fractions. Preliminary work indicates that this may be true of creatinine. If the levels of this metabolite prove to reflect early states of protein malnutrition not yet so severe as to lower total plasma protein, their determination could be of great value.

There are numerous other substances in the blood, the determination of which may in the future prove useful for epidemiological surveys of the type proposed in this report. These include other non-protein nitrogen

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1957, 117

compounds, uric acid and pepsinogen. Certain determinations which are impracticable at present on a survey basis because of technical or other limitations may be used in the future when suitable test methods are devised. This category includes blood-glucose, hormones, etc.

3. USES AND LIMITATIONS OF SEROLOGICAL SURVEYS

The principal objectives of serological surveys have already been outlined: some ideas regarding their application to the study of both current and long-term disease problems are given below.

3.1 Studies of current disease problems

Serological surveys can contribute practical information in terms of current disease problems in a variety of ways, many of which have already been pointed out. Particular attention is directed towards the following:

(1) When vaccination programmes are contemplated—as is the case in many parts of the world today with respect to poliomyelitis—knowledge of the susceptible age-groups in a given population is valuable in designating which persons are to be vaccinated, and in what order of priority.

(2) Serological surveys can yield information on the geographical distribution of infectious agents, which is of prime importance on a world-wide scale in order to anticipate the possible invasion of new areas by particular agents. If such information is available, public health authorities are in a better position to take adequate preventive measures. Furthermore, if the presence in an area of certain agents is known on the basis of serological studies, outbreaks of disease caused by such pathogens can be dealt with more rapidly and effectively.

(3) In the case of repeated surveys, the reappearance of an agent in a population after long absence may be heralded by the appearance of antibodies before the disease becomes clinically apparent.

3.2 Long-term studies

Long-term studies can be accomplished by repeated cross-sectional surveys or by longitudinal surveys. The term “longitudinal survey” is here used to denote a survey in which a sample of individuals is taken, and these same individuals are then re-examined at intervals. In repeated cross-sectional surveys, on the other hand, a fresh sample of individuals is taken on each occasion.

More complicated sampling patterns are of course possible and sometimes useful. In particular, in longitudinal surveys arrangements may be

made to replace losses from the sample as they occur, or some method of systematic renewal may be adopted.

3.2.1 *Repeated cross-sectional surveys*

Cross-sectional surveys, especially when repeated, can yield information on the changing pattern of infection in a community and on shifts in geographical distributions. Moreover, data can be obtained on possible antigenic shifts, as has been observed with influenza viruses. In addition, it may be possible to gather information as to whether previous contact of a population with one or more viruses of a given serological group, such as the arbor viruses, can result in a modified epidemiological pattern when another virus of the same group is introduced in this population.

3.2.2 *Longitudinal surveys*

A more thorough investigation of what may be going on within a given population can be made with sequential samples from the same persons. An ideal to be sought is that of obtaining so-called matched samples of blood from the same individual collected at intervals of months or years and correlated, if possible, with a record of illness covering the contemporary period. This approach is obviously more informative than cross-sectional surveys but is more difficult and time-consuming. Its aim is to relate the frequency of overt and recorded illness to the development of detectable traces of infection in the form of antibodies and to measure the rise or decline or antibody levels in the same individual. To achieve this end it is necessary to sample the population of designated areas by means of periodic visits, at which time medical histories and blood samples are taken, as well as other specimens such as throat swabs, sputum, and faeces, if these seem advisable.

It is, however, necessary to recognize that there are a number of limitations to the value of these surveys and there may sometimes be serious difficulties in their interpretation. The limitations in question are listed below.

3.3 **Limitations connected with antibodies**

3.3.1 *The variability of different infections in terms of persistence of antibody*

Some antibodies last presumably for a lifetime, while others are transient and disappear within a short period of time. Infections with short-lived antibodies—such as the ASLO of streptococcal infections—therefore lend themselves only to special types of survey closely following recent spread.

3.3.2 *The presence of antibodies against an agent which do not necessarily indicate previous infections with that agent*

Infection by an antigenically distinct but closely related agent may result in cross-reacting antibodies, which create a difficult diagnostic problem. In cases in which the cross-reactions are intense, no definite conclusion can be reached concerning the specific etiological agent, unless it is actually isolated in that area. Improved techniques to detect antibody specificity, as in influenza, will no doubt be available in future years.

The above limitations are in part related to the types of test used for the survey. Certain tests may give more specific answers than others—for example, the mouse intracerebral neutralization with arborviruses of groups A and B is usually a more specific test than HAI. The antibody detectable, for example, by the neutralization tests persists for much longer periods of time than that detected by the complement-fixation test, as in poliomyelitis and influenza. However, advantage may be taken of the shorter duration of complement-fixing antibodies in some infections, as a means of detecting recent infection, in contrast with other antibodies which detect remote as well as recent infections.

3.4 **Limitations connected with the tests**

Reproducibility by different investigators and sensitivity of a given test are two examples of such limitations. Furthermore, there may exist more or less marked individual differences in the interpretation of results when gradations rather than completely negative or completely positive responses are observed.

3.5 **Limitations connected with the sera**

The fact that sera from certain species have the capacity to react with some viruses in a manner similar to that of immune sera is another difficulty encountered in serological tests, particularly when dealing with animal sera. This neutralizing effect, simulating the action of specific antibodies, is not related, as far as is known, to any previous contact or exposure to the virus. It would be erroneous to conclude from such neutralizing action that a virus had been at work. An example of this situation is the capacity of some calf sera to inactivate polioviruses.

It is finally to be borne in mind that serological surveys are not applicable to many diseases of great importance, such as tuberculosis, for instance, owing to the fact that no generally usable methods are at hand. Furthermore, the methods that are used for a number of infections are in a continual state of development and improvement. Consequently it is strongly recommended that, in addition to carrying out surveys with available

methods, every effort should be made to promote basic studies for the improvement of available test methods as well as for the development of new ones. In view of the rapidly increasing number of infectious agents which may be included in surveys, the development of micro-methods for immunological procedures using as small amounts of serum as possible deserves a high priority.

4. METHODS OF SAMPLING THE POPULATION

4.1 General considerations

The determination of the parts of the world (countries, groups of countries, or zones cutting across political boundaries) which are to be covered by any particular survey will depend largely on the primary objects of the survey and the interests of those initiating it. The Study Group would, however, urge that efforts be made, as opportunity offers, to cover parts of the world for which as yet there is little epidemiological information.

The method of selecting the sample of individuals for examination from within the zone to be covered by the survey depends in part on the objectives of the survey and in part on the practicability of selecting and examining different types of samples and the relative ease with which this is carried out.

If the main objective of the survey is to obtain an estimate of general levels of infection or immunity for the population of the whole of the zone, or of a political subdivision of it, the ideal method of sampling would be to select individuals at random from all localities in such a manner that each locality would be represented in the sample in proportion to the population of that locality. Such a method is, however, quite impracticable because of the difficulties of selection and of examination. Such a sample, if obtained, would also have the serious disadvantage that the characteristics of small groups of the population which were of particular interest would not be well determined, since the numbers of the sample falling in these groups would be very small.

From the practical viewpoint, therefore, it is essential to confine the sampling to a few relatively small areas (which may be villages or groups of villages, towns or parts of towns, etc.) with a corresponding increase in the numbers sampled in these areas. The first question to be settled is how these areas should be selected.

There are two main possibilities. The areas for sampling may be selected by a random process, in such a manner that all areas have equal (or more generally unequal but pre-determined) chances of inclusion; or deliberate choice may be exercised so as to obtain areas which are regarded as typical of the various parts of the zone or are of special interest for other reasons.

Sampling of the first type is known technically as multi-stage sampling. The sample of areas constitutes the first stage of the sampling process; the sampling of individuals within the selected areas the second stage.

4.2 Random selection of areas

There are various ways of selecting the areas which would satisfy the requirements necessary to give a statistically valid sample, capable of furnishing objective estimates for the whole population. It is, for example, permissible to subdivide zones into regions which are believed to be relatively homogeneous in the characters under investigation (stratification by regions), and to sample from within these regions. If the characters under investigation differ markedly from region to region, this will give increased accuracy in the sample. It will also ensure that all regions are adequately represented in the sample; if fully random sampling is used, it may by chance happen that some regions are poorly represented in the sample.

If certain further statistical requirements are satisfied in the sample structure, it is possible to make rigorously valid estimates of the sampling errors. Even if this is not done, adequate estimates of these errors can usually be made. The details of the procedures to be followed are described in the standard statistical textbooks on the subject.

4.3 Selection of typical areas

Many variants are possible, depending on circumstances. In a predominantly rural economy, with village communities, for example, in which there are several contrasting geographical regions, the parts of the zone which are typical of these regions might first be demarcated, excluding the transition and fringe areas entirely. Villages within these typical areas might then be selected at random, or the villages might first be classified according to tribal characteristics, distance from lines of communication, large towns, etc., and a random selection made of these; or a deliberate choice might be made with these characteristics in mind. In addition, there will probably be one or more large towns and also a number of smaller towns which require sampling separately. To effect this, typical areas of the large towns and some of the more typical of the small towns may be selected.

A sample of this type must not be regarded as a valid statistical sample of the whole zone, and any estimates of the average incidence of given conditions for the whole zone which are derived from it must be recognized as being of uncertain accuracy. Nevertheless the information provided may be quite adequate to give a reasonable picture of the general situation throughout the zone, and the contrasts between the different types of area which become apparent are likely to be much more clear-cut and illuminating than is the case when a random selection of areas is made, since many

such randomly selected areas are likely to be of the transition type, where the situation is necessarily confused. If, for example, the incidence of a condition is similar for the populations of all the selected types of area, it is reasonable to conclude that it is much the same for the whole zone, while if it differs markedly, the variations are likely to be of more interest than any precise determination of the average value for the whole country.

From the above it will be clear that there are marked differences between the sampling requirements of a survey of this kind and sampling for a population census or an agricultural census of a country. In these latter surveys accurate estimates of the total population and the total agricultural production respectively are required, and consequently rigorous sampling procedures covering the whole country must be employed.

It will be noted that it has been suggested above that random sampling may be resorted to for the selection of areas within typical regions. Such a method of selection is a useful safeguard where there are no relevant considerations which dictate the selection of certain areas rather than others.

Frequently there may be strong arguments for including a certain small town, say, because a good deal of relevant background information is available for it. This is quite legitimate, but it should be recognized that the existence of such information may imply that the town is atypical. It is wise therefore not to confine the selection to such towns.

It is also a useful precaution to include more than one area from among those which are believed to be typical of a given set of conditions. The results obtained from two or more such areas provide valuable evidence of the extent to which the areas are really similar and are a safeguard against the misinterpretation of anomalous results caused by, for example, a recent local epidemic.

4.4 Selection of samples of individuals from selected areas

In selecting individuals for examination from within the selected areas rigorous sampling procedures must be adopted, particularly for a multi-purpose survey. Casual methods of selection which may serve to reveal a clear-cut situation, such as that in which virtually the whole of the population, except the very young, carries antibodies against poliomyelitis, can give a very misleading picture of, say, the genetic characteristics of the population.

In general the best way of selecting a random sample of individuals is by the enumeration of the dwelling-places of the selected area and the random selection of households from the list thus prepared. The inclusion of whole households, with a record of the particulars relating to them, is also advantageous in the investigation of certain characteristics, in particular the study of genetic and nutritional factors and of the incidence of certain infections. The various procedures which may be followed

have been frequently described. The one chosen will depend on local circumstances.

It is in general important to ensure that the sampling from within age-groups is properly random. (The question of age-groups is discussed in more detail below.) Consequently such practices as that of including only families with children in order to secure a greater proportion of children should not be adopted. Childless households should be given their due chance of inclusion in the sample.

If separate samples from two different socio-economic groups in a large town are required, this can often be done on an area basis, different areas being selected to provide samples of the two groups. In such cases members of the population belonging to socio-economic groups other than that being sampled can be excluded. In sampling small towns and rural areas, it will probably be best not to attempt to separate the different socio-economic groups. If their characteristics require to be evaluated separately, this can be done when the data are analysed.

An alternative to sampling by households is to base the sample on institutions such as clinics and schools and organizations such as the army, factories, etc.; it is often easier to sample from institutions and organizations than by households. Such samples are particularly useful when interest centres on particular age-groups of the population (e.g., school-children), but it must be recognized that such groups are often not fully typical of the corresponding age-groups in the population as a whole. Thus in places where, say, only 50% of the children of a given age are attending school, these 50% are likely to differ considerably in socio-economic status and possibly also in other characteristics such as race from the remaining 50%.

Special sub-groups (e.g., of pregnant women) may be added to the sample if desired. Such groups must be clearly differentiated in the records from the main sample. Special methods of inquiry will be required to locate individuals eligible for inclusion in such groups. The methods of selection adopted should be effectively random.

4.5 Treatment of age-groups

Since the epidemiological characteristics of individuals vary greatly with age, and the differences are themselves of paramount interest in many studies it is important that all age-groups should be adequately represented in the sample. Frequently it is desirable to include a greater proportion of the younger age-groups, because changes are taking place at a more rapid rate in these groups.

One method of obtaining a sample with the required number in each age-group would be to stratify (i.e., group) the population to be sampled by age, and select the required number at random from each age-group. This method, however, has two serious disadvantages. In the first place

it requires a preliminary listing of the whole population of the area to be sampled, with their ages—a procedure which is very laborious if the number is at all large. In the second place simple random selection from within age-groups will not give a sample which contains many whole families. Other methods have therefore to be sought.

If the age-group distribution required is that of the general population (i.e., no additional representation of the younger age-groups is required), random sampling by households or individuals will produce a distribution in the sample which approximates to this distribution. However, there will be chance variations due to the random sampling, and there may also be real differences in the age-group distributions of the populations of the different areas, and these will affect comparisons between samples from different areas based on the mean values over all age-groups of the measure under consideration. A method of eliminating this source of variation in the course of the statistical analysis is described below. This, however, involves troublesome calculations which it may be considered desirable to avoid.

The alternative is to take steps, when selecting the sample, to arrange that the age-group distribution of the sample conforms to some standard pattern, which may be approximately that of the general population or may contain a greater proportion of the younger age-groups. The simplest method is to take all individuals as they are selected and include each in the sample until the quota of the age-group to which the individual belongs is full. This procedure, however, is not fully satisfactory if a greater proportion of the younger age-groups is required. If, for example, the sample of households from an area or group of areas of a town is being examined systematically (after initial random selection), the households last dealt with, which will be situated in one part of the town, will contribute only children to the sample. This is obviously undesirable and in particular will introduce lack of comparability between the younger and older age-groups. This difficulty can be overcome by rejecting a proportion of the older age-groups throughout the course of the sampling. Thus if the proportions required in the sample of children aged 0-4.9 and 5-9.9 are three times and twice those occurring in the population, one out of three children aged 5-9.9 and two out of three of older people should be rejected. Final balancing up of the required quotas can be made as above. The methods of rejection followed should be such that whole families are included to the maximum extent possible. In certain cases a preliminary selection of a somewhat larger number of households than will ultimately be required, and a listing of all members of these households by age, may be helpful.

When standardizing for age it may also be considered worth while to standardize for sex, so that each age-group contains equal numbers of males and females.

Even when the numbers in the various age-groups are standardized, there will be random sampling variations of the age-distributions within the groups. These may affect comparisons when the measure under consideration varies very rapidly with age. To overcome this it has been suggested that only children of specific ages should be sampled, the intermediate ages being excluded entirely. This procedure is only likely to be practicable when sampling from schools and similar institutions and presupposes that the ages of the children can be determined with reasonable accuracy.

4.6 Adjustment of estimates for inequalities of age-distribution

In cases in which the age-distribution has not been standardized for samples from different areas, and epidemiological factors which vary greatly with age are being considered, substantially more precise comparisons between the mean values over all age-groups for the different areas will be obtained, if these mean values are adjusted to allow for differences in age-group distribution. This can be done by calculating the mean values of each age-group in an area, and calculating a weighted mean of these means, the weights being taken proportionately to the numbers in some standard age-group distribution which should be approximately the average distribution of the samples from all areas. This procedure requires that all age-groups should be adequately represented in every sample, and for this reason, and to simplify the computations, too fine a grouping should not be taken.

If the age-distributions of the samples differ considerably from that of the population, owing to the inclusion of a greater proportion of the younger age-groups, and average figures representative of the population as a whole are required, similar weighted means, with weights corresponding to the age-group distribution of the population, will be required.

4.7 Size of sample

The number of individuals that should be included in a unit sample (village, area of a town, etc.) depends very greatly on the type of information that it is hoped to obtain and on the degree of detail which is required.

In quantal (affected or not affected) determinations, the standard error of the percentage affected in a random sample of n from a large population is given by the well-known formula

$$100\sqrt{\frac{pq}{n}}$$

when p is the proportion affected in the population and $q = 1-p$ is the proportion not affected. This standard error can of course also be expressed

as a percentage of the expected number affected. Typical values for a sample of 100 are :

<i>p</i> %	<i>S.E.</i>	<i>S.E. as % of affected</i>
90	3.0	3%
80	4.0	5%
50	5.0	10%
20	4.0	20%
10	3.0	30%
5	2.2	44%

In roughly two-thirds of all samples the actual errors will be within limits given by the true value plus or minus the standard error, and in roughly 19 out of 20 cases within limits given by the true value plus or minus twice the standard error. Thus, in a population with 20% affected, two-thirds of all samples will give values between 16% and 24% and 19 out of 20 samples values between 12% and 28%. The corresponding limits with 80% affected are 76% and 84%, and 72% and 88%.

The standard errors vary inversely as the square root of the number in the sample. Thus, for samples of 25, the above standard errors will be doubled, and with samples of 400 they will be halved.

If it is necessary to compare the separate age-groups in a sample, the relevant standard errors of the separate age-group percentages will depend on the numbers in the age-groups and will thus be substantially greater than the standard error for the whole sample. Frequently, however, it is possible to combine samples from similar areas for age-group comparisons, with a corresponding increase of precision and, incidentally, a simplification of the presentation of results.

In determination of the mean percentage affected over all age-groups there is a marked gain in precision when the age-groups are standardized and the range of percentages over the different age-groups is substantial. Thus, in a population with 50% affected, composed of five age-groups containing equal numbers with 10%, 30%, 50%, 70% and 90% affected respectively, the standard error of a sample of 100 selected so as to contain equal numbers in the different age-groups (20 in each group) is 4.1% compared with 5.0% for a random sample. In this case a standardized sample will have the same precision as a fully random sample of 1.5 times the size. Similar gains in accuracy will result from standardization of estimates for age in cases in which there has not been standardization of the sample age-distributions. It may be noted that in the above example further subdivision into 10 age-groups with 5%, 15%, 25% . . . 95% affected would not appreciably increase the precision.

Taking all these factors into account, it is considered that a suitable sample size for the population groups between which it is desired to make comparisons is 300-600. The number and age-distribution required to give satisfactory results depends very much on the nature of the problems

under investigation and on the epidemiological situation. Thus for poliomyelitis surveys, samples of 425 distributed over the age-groups as follows have been proposed :

<i>Age-group</i>	<i>Number</i>
6/12-1	25
1	25
2	25
3	25
4	25
5-9	50
10-14	50
15-19	50
20-29	50
30-39	50
40 +	50
	425

Indeed, in areas in which practically all but the very young have poliovirus antibodies, substantially smaller samples have been used with success. Since, however, in a multi-purpose survey the blood samples may be needed to give information on many points, some of which may not be at all clear-cut, a fair-sized sample should normally be taken. If for a given purpose a smaller sample appears adequate, the number of laboratory determinations can be reduced by sub-sampling.

In deciding the size of sample required for any given purpose, the magnitude of laboratory and other non-sampling errors that are likely to affect the results should be borne in mind. There is no point in using very large samples when, for example, there are known to be differences between the determinations made by different laboratories which are jointly working on the survey, or when these determinations vary markedly with time, unless special steps can be taken to eliminate such errors by the interchange of samples, etc.

4.8 Records of sampling procedures

It is very important, when compiling the records of the survey, to describe the sampling procedure that has been followed. Detailed descriptions of the boundaries of the areas that have been actually sampled should be placed on record, so that the areas can be identified if a re-survey at a later date is required to assess what changes have taken place. The methods used for the sampling of individuals within the selected areas should also be described.

4.9 Sampling of animal populations

The collection of animal sera in connexion with the serological surveys considered in this report is necessary to complement the information

gained from the human serum specimens with respect to animal reservoirs of infection for humans, rather than for an investigation of animal disease *per se*. There is therefore no need to apply the stringent techniques for random sampling discussed for human populations.

Several limitations are immediately apparent when considering the selection of animals and the numbers which could be sampled. These considerations include such factors as the limitation of numbers and opportunity with respect to various types of wildlife, and the relatively young age-range of domestic livestock, which are usually slaughtered according to economic dictates. Nevertheless, when surveys in particular areas are planned every effort should be made to obtain an adequate number of serum specimens, if possible from representative cross-sections of local animal populations, including those having different degrees of contact with humans—for example, free-roaming swine as compared with those kept in enclosures, range cattle and confined dairy cattle, wandering flocks of sheep and goats and flocks restricted to one locality, urban and rural dogs, poultry flocks left at large and confined flocks. With respect to wildlife the possibilities of obtaining adequate numbers of samples from individual species are not great, so that every advantage should be taken to collect the largest number of specimens possible, utilizing the efforts of various groups already engaged in different wildlife studies (e.g., bird migration and other natural ecological studies, trapping operations to reduce wildlife). Where selection of different wildlife species for blood samples is possible, priority should be given to those species harbouring ectoparasites common to one another and also perhaps found in man.

The advisability of obtaining animal serum specimens from wildlife under conditions of natural foci of infection where human population may be sparse should not be overlooked. Information derived from such studies has often been very useful in the past in clarifying the natural history of some human infections.

5. TECHNICAL CONSIDERATIONS REGARDING THE METHODS OF COLLECTION, SHIPMENT AND TREATMENT OF BLOOD FOR MULTI-PURPOSE EXAMINATION

5.1 Collection of human-blood specimens

Vacutainer tubes provide the most satisfactory means of collecting blood under sterile conditions in the field. It is estimated that for the tests now contemplated a total of approximately 26 ml from each person would be required. In the event that it would be impracticable or inadvisable to obtain this amount from single individuals (e.g., certain young infants),

the population sampled could be enlarged so as to ensure that enough blood is collected.

The approximate amounts of blood required for various purposes is estimated to be as follows :

Oxalated (or citrated) blood	6 ml	{ Whole blood : 0.5 ml—blood groups ; 0.5 ml —haematology Plasma : * 1.0 ml or more—biochemical tests Packed red blood cells : * 2.0 ml—haemoglobinopathias
Clotted blood	20 ml	

* These two fractions are obtained from the same sample—i.e., the packed red cells from approximately 5 ml of oxalated blood and the plasma from the same specimen.

In addition, a blood smear on a glass slide is required for the study of the morphology of red cells.

Since two types of specimen are needed—oxalated (or citrated) and clotted blood—the simplest method of collection would be to use two different sized vacutainer tubes with an adapter¹ for multiple sampling. By this means, with a single venipuncture, 20 ml of clotted blood can be collected in one tube, and 6 ml of oxalated (or citrated) blood in another.

The oxalated (or citrated) sample will be completely used in the set of contemplated tests. On the other hand, of the serum collected (approximately 10 ml) only a small amount will normally be used soon after collection, the larger portion being stored for future studies in years to come. In fact, the establishment of a serum bank for later studies is one of the principal purposes of these serological surveys. The amount to be used in the original tests may be as little as 0.5 ml.

It is anticipated that the actual collection of sera may be done either by specialized personnel attached to certain WHO teams already in the field (tuberculosis survey units, yaws teams and others) or by arrangement with co-operating laboratories, epidemiologists, local health authorities, etc.

5.1.1 *Haematological determinations*

The total haemoglobin and haematocrit must be tested immediately at the site of collection. For other determinations, the specimen should be centrifuged and divided into plasma and packed red cells, the latter being shipped by air immediately (at 4°C) to the appropriate laboratory.

5.1.2 *Biochemical determinations*

The separation of red cells and plasma having been carried out at the site of collection, the plasma fraction should be shipped frozen on dry ice,

¹ Such as is manufactured by the Beckton-Dickinson Company

or, if this is not possible, at temperatures of from -5° to -10°C , obtained by mixing ice and salt. Where possible, total plasma proteins should be determined at the original processing laboratory.

5.1.3 *Immunological studies*

For these determinations, the serum should be separated from the clot on the day of bleeding, the temperature being kept as low as possible, without freezing. Once the serum is separated it should be shipped under refrigeration, but not frozen, for processing at a designated laboratory. Blood transfusion service kits may be useful for this stage of transportation.

The question of how to store the serum requires further research, as there are practical as well as theoretical considerations to be taken into account. It is urgently recommended that a co-operative study on methods of preservation and their effect on the preservation of antibodies in sera be carried out as soon as possible. Undoubtedly the best way at present available is quick freezing and storage at -70°C or lower. As judged from available information, even the more sensitive of delicate systems in a serum (complement, properdin, accessory factors) stand this treatment. On the other hand, this type of storage for long periods of time and on a large scale is not considered feasible. Storage of serum at higher temperatures presents some theoretical as well as experimental drawbacks. According to Greaves,¹ "Serum is not completely frozen at temperatures above -60°C . . ." There is, then, the possibility that even at temperatures between -40°C and -60°C , over the long period of time contemplated, layering of proteins and denaturation with possible loss of antibody potency may occur. It is well known that storing at temperatures around -20°C as in an electric freezer, does result in layering and denaturation of serum proteins. For these and other considerations, such as the danger of mechanical breakdown, it is deemed that storage in the frozen state at the low temperature required to ensure permanence of unchanged serum protein and antibody titres is not practicable.

Another possibility is lyophilization. It is recognized that this process, even when carried out with optimal precautions, may damage certain of the delicate elements that play a role in serological reactions—for example, accessory factor in the neutralization test. Complement is also damaged to a certain extent; therefore it may well be that some damage may be done to specific antibodies. Furthermore, the conditions for optimal lyophilization must be adhered to rigorously, and the initial cost of this type of processing would be high. Nevertheless, the conditions for keeping lyophilized sera are much easier to comply with (storage at 2°C — 4°C may

¹ Greaves, R. I. N. (1954) Theoretical aspects of drying by vacuum sublimation. In: Harris, R. J. C., ed., *Biological applications of freezing and drying*, New York, p. 92

suffice) than if the sera were kept at -76°C in the frozen state. The ease of shipping and handling is so great that lyophilization seems to be the method of choice. The possibility of keeping sera in sealed ampoules at 1°C , never frozen, may deserve investigation. However, owing to the general lack of information on this latter method, it cannot be advocated at the present moment.

No matter what method of preservation is used, the serum should be divided in 0.5 ml or 1.0 ml aliquots, each kept in a sealed neutral glass ampoule. Standard sera of known antibody content should be stored under the same conditions as the sera collected so as to enable an estimate to be made of the deterioration that might have taken place during prolonged storage.

5.2 Collection of animal-blood specimens

The collection of animal sera is governed by technical considerations similar to those involved in the collection of human sera, but it is likely to be difficult to achieve the same standard as that adopted for human sera. A minimum standard is defined as allowing not more than 24 hours to elapse between collection and the removal of serum from clotted blood. Serum should then be stored at 2°C — 4°C until frozen or lyophilized.

Note should be taken of the problem of inter-continental transport of animal sera. Many countries (the USA, the United Kingdom, Australia, for example) restrict the importation of animal sera from other areas. A knowledge of these restrictions should be a prerequisite of widespread studies.

6. SUGGESTED LIST OF ITEMS OF INFORMATION TO BE RECORDED IN SURVEYS

It is clear that much careful study is required before precise details can be given of the information which should be collected in the different types of survey. However, the broad headings under which special items of information should be recorded are given below, followed by some general explanatory remarks.

- (1) Identification of the person bled ;
- (2) Items of epidemiological or statistical value
 - (a) concerning the person
 - (b) concerning the household
 - (c) concerning the community or locality ;
- (3) Concerning the blood drawn
 - (a) Data recorded at the source
 - (b) Data recorded in the testing laboratory.

6.1 Identification of the person bled

Name, name of head of household, address, locality, in the case of children the name of the school attended, serial number of the household or individual used in connexion with sampling procedure. Code number of the person for cross-reference with blood sample.

6.2 Items of epidemiological value

(a) *Concerning the person*

Age (the reliability of the information should be noted), sex, marital status, socio-economic status, relationship of the person bled to the head of the household, ethnic origin, religion, height, weight, previous immunization record, occupation. In the case of females, whether pregnant, lactating, etc.

(b) *Concerning the household*

Number and ages of all household members, with reference number if bled, socio-economic status, means of livelihood, living conditions, collective living (schools, barracks, etc.), contact with animals.

(c) *Concerning the community surveyed*

Whether urban or rural, size of community, main occupations of the people, main industry, location and facilities for communication (for rural populations), medical facilities available, special remarks about prevailing dietary habits, remarks about housing and sanitary conditions, animal accommodation in the house or outside, kinds of pet kept in households, community exposure to disease vectors, whether blood samples were collected through a statistical sampling procedure of the whole community or from a school, baby clinic, industrial group, hospital, accident clinic.

6.3 Blood drawn

(a) *Data recorded at source*

Code number of blood sample to relate it to individual; quantity of blood drawn, the date of bleeding.

(b) *Result of laboratory examinations*

Date, nature of the tests carried out, results, name of individual undertaking the test.

The record cards should be designed to facilitate machine processing. A brief manual of instructions should be prepared to guide the team in the completion of the record card.

6.4 Animal sera

For animal sera an abbreviated catalogue would be employed. The essential information is :

- (1) Species
- (2) Breed
- (3) Sex
- (4) Age (under 1 year, 1-5 and over 5 years)
- (5) Agricultural purpose (dairy, meat, etc.)
- (6) Whether usually housed or left at large
- (7) Animal movements.

7. CRITERIA FOR RELEASE OF SPECIMENS

7.1 Human sera

It is recommended that, because of the great potential value of the stored specimens in future years, the release of these specimens should be carefully restricted. Each request for serum specimens should be subjected to thorough scrutiny by specialists in the proposed field of study. The Study Group suggests that the Director-General should establish in due course an appropriate mechanism within the WHO Secretariat to act in consultation with experts, who will advise upon all requests.

7.2 Animal sera

It should be recognized that while these sera are held for primarily medical purposes, there may be circumstances in which WHO would be justified in releasing serum for investigation of diseases primarily of animal interest. In these instances the advice of experts nominated by FAO might be of value.

8. STANDARDIZATION OF TESTS

This is a continuing activity of WHO and a great deal has been accomplished by various Expert Committees including especially that on Biological Standardization. (Reference should be made to the relevant reports in the *World Health Organization Technical Report Series*.) Serological tests are, however, in a continual state of development and when new modifications or new tests are used, investigators should be encouraged to describe their precise methods in considerable detail.

The provision of standard reagents—both sera and virus strains—will be a function of central reference laboratories, set up under the proposed expanded programme of research. However, periodic checks on the comparability of results of different laboratories are advisable. These should be carried out by the distribution of unknown sera for testing, the results of tests to be compared with those of other laboratories testing the same specimen.

Standard samples of certain rare abnormal haemoglobins should be stored frozen, so that they may be available as reference specimens.

The Study Group strongly emphasized the importance of standardization of all tests.

9. RECORDING AND REPORTING OF RESULTS

The results of tests carried out with blood samples or sera supplied from both international and national collections should be promptly reported to WHO, and, where appropriate, to FAO. As the methods used by the individual workers may vary to some extent, it is recommended that a complete description of the methods employed be sent with the results of the tests. These results should be made available to the local health authorities at the same time. The results will be considered as restricted to interested authorities and individuals until their publication by the investigators concerned.

In order to make the information derived from these surveys available to interested individuals at an early date, WHO will bring together and circulate this information in the form of reports to be issued at appropriate intervals.

A system of recording the results of tests must be put into operation, so that the results of all tests will be available on each sample of serum existing in the serum bank. This should be undertaken centrally, and it is clear that the handling of the mass of data which will accumulate will require the adoption of a punch card system.

10. RECOMMENDATIONS FOR THE DEVELOPMENT OF MULTI-PURPOSE BLOOD SURVEYS AND OF AN INTERNATIONAL COLLECTION OF SERA

The Study Group concluded that the multi-purpose approach to immunological and other surveys outlined in this report is a practical proposition and would undoubtedly result in important contributions to the study and control of many human diseases. In particular it was held that the long-

term aspects of these proposals are highly significant from the point of view of world health, and that over the years important results will be produced. Because of these long-term aspects international collections of sera under the administrative control of WHO are regarded as essential.

There are certain logical steps which must be taken in establishing these collections of sera and in initiating world-wide studies of other substances in blood. The first step is to determine whether the technical procedures outlined in this report are applicable in practice, and, in particular, what modifications are necessary to facilitate their application and to increase the precision and value of the results. This can only be done by pilot studies in the field under different conditions. It is therefore strongly recommended that WHO, with the aid of its existing field survey teams (tuberculosis, venereal diseases and treponematoses, etc.), should undertake such pilot studies as soon as possible. These pilot studies should not in the first place be too large, so that the demands for laboratory tests and the burden on the field teams can be kept within bounds. A relatively small number of tests under different circumstances will show how the ideas put forward in this report will actually work in practice.

Provision must be made for the early examination of the material collected in the pilot studies in order to determine whether the methods of collection and shipment are satisfactory for the tests proposed. Contact should therefore be established with laboratories experienced in the various tests to enlist their collaboration in comparative studies in which, for preference, tests on the same material should be carried out simultaneously by different laboratories.

In the proposed pilot studies attention should be given to the suitability of currently available apparatus and blood-collecting kits with a view to stimulating the production of better apparatus, should this prove necessary.

Pilot studies should be initiated at once to determine the stability of various antibodies under the conditions of collection, processing and storage proposed in this report.

It is therefore recommended that the problem of providing for the rather extensive laboratory facilities required for larger surveys be tackled without delay. Here the most urgent and important provision is for facilities for processing and storing sera in strategically situated laboratories all over the world. Suitable laboratories must be identified, their co-operation enlisted and the necessary equipment and, probably, additional technical assistance provided for.

The establishment of methods of recording the surveys is of primary importance. The suggestions given in this report are preliminary in nature, since the precise information to be collected and the design of forms for its recording, taking into consideration the indispensability of transferring this information to punch cards, are matters for careful study which should be initiated as soon as possible. It is of course essential to ensure that

some central collecting unit for these records be established with the proper facilities for processing cards, keeping them up to date as tests are performed, analysing the data obtained and providing regular summaries of the results. WHO should take the responsibility for this.

The Study Group noted that under the proposed expanded programme of virus research a very high priority had been given to the production of standard laboratory reagents. It wished to place on record its entire agreement with this recommendation, which is regarded as essential to the development of a programme such as is envisaged in this report. It is also pointed out that such reagents will be required for many bacterial and other infections. WHO should consider how these reagents may be obtained.

It has already been pointed out that, in the first instance at least, the stimulus for a survey will arise from some immediate health problem. WHO should therefore make inquiries as to whether surveys are being planned in countries throughout the world and, if so, endeavour to enlist the interest of the appropriate authorities in applying the broad techniques outlined in this report. However, the Group wished to place on record its view that a specific local interest should not be regarded as essential.

The multi-purpose survey approach is of such potential importance that it is considered suitable for adoption and implementation as a central activity during the Public Health and Medical Research Year proposed by the United Nations.

WORLD HEALTH ORGANIZATION : TECHNICAL REPORT SERIES

	Price		
	s. d.	\$	Sw. fr
<i>Recent and forthcoming reports :</i>			
No. 165 Expert Committee on Plague Third report	1/9	0.30	1.—
No. 166 Effect of Radiation on Human Heredity First report of the Expert Committee on Radiation			<i>In preparation</i>
No. 167 Public Health Nursing Fourth report of the Expert Committee on Nursing	1/9	0.30	1.—
No. 168 Hypertension and Coronary Heart Disease : Classification and Criteria for Epidemiological Studies First report of the Expert Committee on Cardiovascular Diseases and Hypertension	1/9	0.30	1.—
No. 169 Joint WHO/FAO Expert Committee on Zoonoses Second report	3/6	0.60	2.—
No. 170 Expert Committee on Respiratory Virus Diseases First report	3/6	0.60	2.—
No. 171 Mental Health Problems of Aging and the Aged Sixth report of the Expert Committee on Mental Health	3/6	0.60	2.—
No. 172 Expert Committee on Biological Standardization Twelfth report	1/9	0.30	1.—
No. 173 Methods of Radiochemical Analysis Report of a joint WHO/FAO Expert Committee	5/-	1.00	3.—
No. 174 Expert Committee on Hygiene and Sanitation in Aviation First report	3/6	0.60	2.—
No. 175 Preventive Aspects in the Teaching of Pathology Seventh report of the Expert Committee on Professional and Technical Education of Medical and Auxiliary Personnel	1/9	0.30	1.—
No. 176 Role of Hospitals in Ambulatory and Domiciliary Medical Care Second report of the Expert Committee on Organization of Medical Care	1/9	0.30	1.—
No. 177 Social Psychiatry and Community Attitudes Seventh report of the Expert Committee on Mental Health	1/9	0.30	1.—
No. 178 Requirements for Biological Substances : 1. General requirements for manufacturing establishments and control laboratories 2. Requirements for poliomyelitis vaccine (inactivated) Report of a study group			
No. 179 Requirements for Biological Substances : 3. Requirements for yellow fever vaccine 4. Requirements for cholera vaccine Report of a study group			<i>In preparation</i>
No. 180 Requirements for Biological Substances : 5. Requirements for smallpox vaccine Report of a study group			
No. 181 Immunological and Haematological Surveys Report of a study group	1/9	0.30	1.—
No. 182 Iron Deficiency Anaemia Report of a study group	1/9	0.30	1.—