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PHILIPPINES PHARMACEUTICAL INDUSTRY DEVELOPMENT STUDY

DP/PHI/87/019

PHILIPPINES

Technical report: Biotechnology for Production of Diagnostics in the Philippines*

Prepared for the Government of the Philippines
by the United Nations Industrial Development Organization
acting as executing agency for the United Nations Development Programme

Based on the work of F. Peterfy
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United Nations Industrial Development Organization
Vienna

* This document has not been edited.

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1 . SUMMARY

In the field of antibody based diagnostics there are a lot of talented people in the Philippines,and they have promising accomplishments. The situation is ripe for industrial development.

It is advisable to give priority to two projects: the Hepatitis B test and the blood typing sera. These products are important for the health sector of the country and a sizable market exists for them. The production technology of Hepatitis B test is a sophisticated one, but it is over the R&D stage and the product is ready to be introduced to the market. The manufacture of blood typing reagents can be started in a year time using imported bulk sera as raw material, than this sera may be substituted by domestically produced immune sera or monoclonal antibodies. This two projects would give a boost to the development of the Philippine biotechnology.

The calculated immediate need for Hepatitis B testing is 1,440,000 per annum, and the expected turnover of the domestic product is 300,000 tests. The cost of production would be around P/2 per test, and a realistic price may be P/15 per test. A similar imported product costs P/29 per test. As for investment, the total sum for equipment and

animal houses is roughly $ 200,000 and additionally a laboratory area of 100 square metres is needed.

A private firm should pay a price for the transfer of technology. The participants of the project (Research Institute for Tropical Medicine and the University of Philippines' Liver Study Group)may sell the know how for a certain sum or/and for royalty payments. The investment costs might be too high for a small diagnostic firm. The burden of investment can be reduced by several arrangements: (i) step by step transfer of technology, (ii) leasing of laboratory space, animal house, equipment.

About 1,500,000 blood typings are required in a year, and it means, that 90 litres are needed from the different typing sera. A domestic producer may expect a total turnover of 100 litres per annum. It is recomended to proceed in successive steps: (i) The first step may be the installation the facilities for sterile filtration, distribution and quality control. It is possible to purchase ABO typing sera in bulk for $ 0.2-0.5/ml in the world market, and the cost of production would be about P/1 per test. The cost of basic equipment is roughly $ 25,000. (ii)The next stage is the local production of reagents, and two distinct possibilities exist: collection of human sera with good antibody titres and production of

monoclonal antibodies in cell cultures. In the first case it is possible to produce a test at a cost of P/0.5, at an investment of $ 30,000. The cost of production of the monoclonal antibodies would be half of that, but a grant for R&D is required at the value of $ 500,000 (a project for fetal calf serum production is included). This project would give a big boost to the development of modern biotechnology.

The producer of Hepatitis B test and/or blood typing reagents may be public or private. A laboratory under the DOH might serve the needs of the public health sector. It represents 24% of the market, and it is too small for a producer to be viable economically. This laboratory will need a continuous financial support. A small private firm should be supported too at the start [technology transfer at cut-rate price, reductions in import tariffs or/and income tax, soft loans, etc], but later it might generate profits, and develop new diagnostics.

For the sake of future it is recomended to concentrate the R&D activities in the human health sector to the diagnosis of schistosomiasis and malaria and in the agriculture to the detection of plant diseases and toxins.

2. INTRODUCTION

Biotechnology is a technology based on biological processes. This broad-based definition includes traditional processes such as fermentation, brewing and cheese manufacture, as well as powerful new techniques like genetic engineering.

The application of biotechnology is as old as the history of mankind: already over 5000 years ago man knew how to prepare beer, wine, bread and cheese. However, it was not discovered until the middle of the 19th that the products described above were the results of fermentation processes involving live organisms, namely microbes.

Biotechnology is based on many fields of basic science. Molecular biology holds a central position, and the discovery of the structure of DNA in 1953 can be seen as the basis for modern biotechnology, that can be defined as the application of basic science and egineering to biological processes, with the aim of creating products and services.

Genetic engineering may be defined as the manipulation of hereditary material by various molecular and cellular

techniques such as recombinant DNA and cell fusion. The specific technique of DNA recombination allows introduction of a foreign gene, which may be of human origin, into the DNA of bacteria, yeast or other cells.Monoclonal antibodies were first commercialized in 1980 and a recombinant DNA veterinary vaccine was launched in 1982. Presently a great number of commercially valuable proteins, such as interferon, insulin, human growth hormone and tissue plasminogen activator produced by this method, have now been approved for human use.

The application of the new technology has so far been developed furthest in medicine and veterinary medicine. The research is still highly active, with the expectation of new diagnostic methods, better and cheaper antibiotics, vaccines against viral and parasitic diseases, new therapeutics for cardiovascular diseases, cancer, etc.
The consumption of recombinant DNA products is not high in the Philippines presently. According to IMS Philippine Hospital and Drug Store Pharmaceutical Audits 2.3 million units of recombinant human insulin were purchased for P1,213,000 in 1987. Import data for interferon were extracted from the issues of Business Satistics Monitor, January 1987-June 1988. During that period 3.092 million units of recombinant interferon were imported for $23,628.

As for diagnostics no data is available on consumption but several products based on modern biotechnology are used in the Philippines (blood typing reagents, ELISA kit for HIV antibodies).

The modern biotechnology provides new possibilities at other areas too, but this report will focus on the field of diagnostics. To give a general picture the prospects for development are listed below:

In medicine and veterinary medicine:

-diagnostic products

-new and/or cheaper antibiotics

-hormones, growth factors, enzymes, etc.

-vaccines

In agriculture and forestry:

-diagnostic products for plant diseases

-development of plants wich can utilize atmospheric nitrogen

-development of varieties resistant to disease, pests and severe growth conditions

-improvement of the material propeties and utilization of plants and animals

-feed production from waste material

-improvement of breeding of animals

In industry:

-enzyme technology

-biosynthesis of substances

-improved efficiency in recovery

-extraction of metals from ores

In environmental care:

-destruction of wastes and poisonous substances

-utilization of waste

Diagnostics were the first commercial products based on modern biotechnology.In the field of diagnostics a substantial part of the total market is taken by products based on antibodies. The traditional biotechnology for antibody production is the immunisation of large animals or human beeings and the antibodies can be extracted from blood drowned. The quality and specificity of the traditional polyclonal antibodies are changing from animal to animal, from bleeding to bleeding. Such reagents were replaced in some diagnostic kits by monoclonal antibodies at the advent of modern biotechnology.In 1975 Kohler and Milstein developed the hybridoma technique which allowed

for the first time the in vitro production of antibodies recognising specific antigens of choice (Fig.1). The exquisite specificity of monoclonal antibodies combined with the potential for producing them reproducibly in unlimited quantities has lead to their widespread application in many areas.But the pros and cons should be considered on case by case basis for monoclonal and polyclonal antibodies, because in a lot of cases the traditional ones work better.

Diagnostic agents were put into the first group in the list of priorities by the Department of Science and Technology (See: Annex II). Different development patterns can be considered for the Philippines:

-production based on traditional biotechnology

-start with kits based on polyclonal antibodies than replace them with monoclonals

-production based on modern biotechnology.

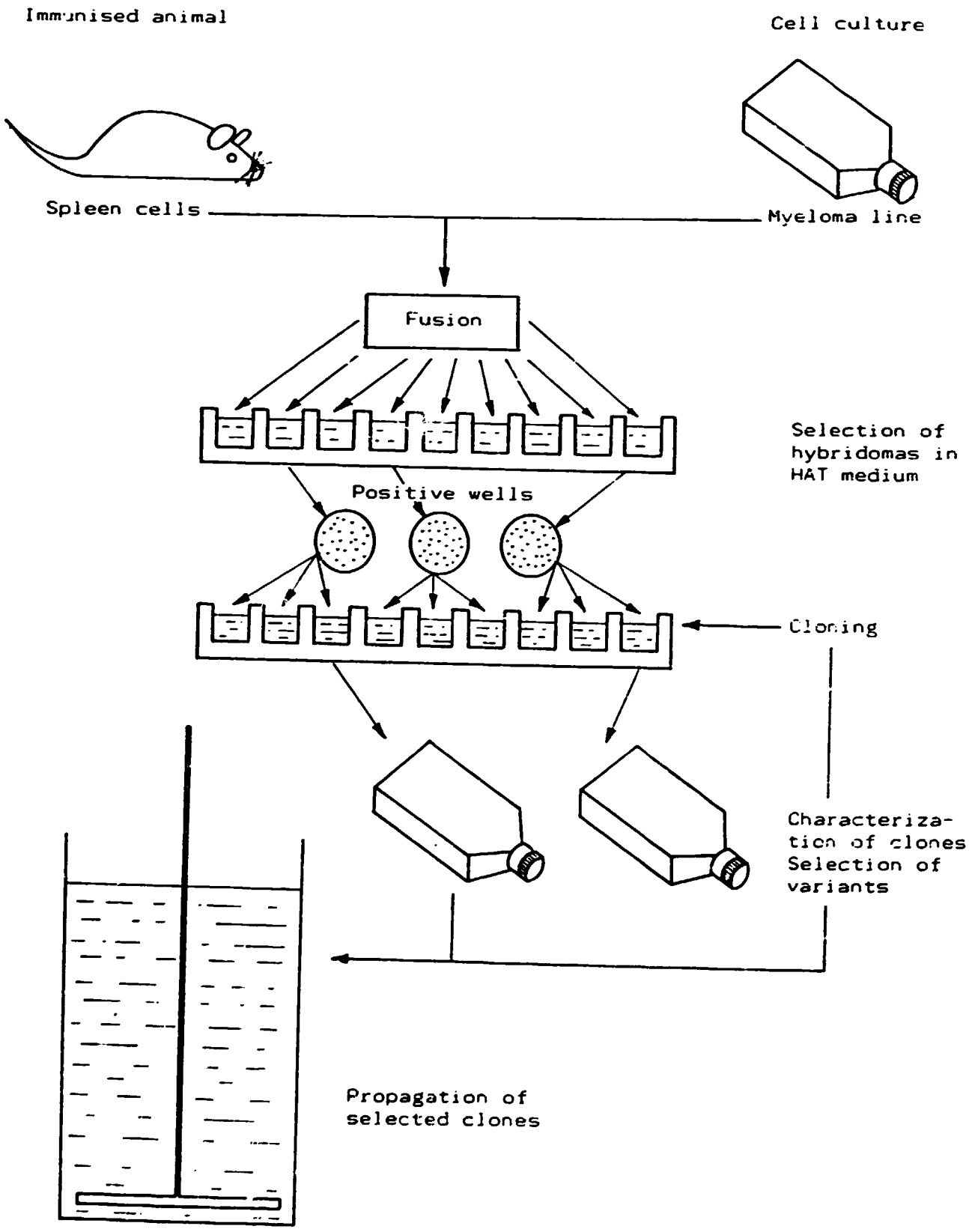

Fig. 1. Diagram of the procedure for the derivation of hybridomas

3.RESEARCH AND DEVELOPMENT ACTIVITIES IN THE PHILIPPINES IN THE FIELD OF ANTIBODY BASED DIAGNOSTICS

In the Philippines I have met a lot of intelligent, well-trained people, I have found laboratories with very good equipment and I have seen some promising results. It is high time to exploit the human resources more effectively for the benefit of the country. This short survey focuses on one segment of biotechnology : the antibody based diagnostics to illustrate that the situation is ripe for further development. The laboratories where this reagents and diagnostic kits were used as tools but not prepared or improved are not included in this list.

3.1.Research Center for Natural Sciences, University of Santo Tomas

anuel D. Navarro, Fortunato Sevilla, Beatrice Q. Guevara

A method based on extraction, anion exchange
omatography and latex agglutination was developed for estimation of urinary beta HCG. It is an important marker of malignancy in the absence of pregnancy.

3.2.Department of Parasitology, College of Public Health, University of the Philippines, Manila
Edito G. Garcia, Wilfred U. Tiu

Monoclonal antibodies were produced against the antigens of Schistosoma japonicum, and they were used in ELISA test systems.

3.3.Research Institute for Tropical Medicine
Mediadora Saniel, Augusto L. Lingao, Remigio Olveda

A reverse passive hemagglutination test was developed for the detection of HBsAg. The Institute has animal house and facilities for monoclonal antibody production.

3.4.Molecular Biology and Biotechnology Program, College of Science, University of the Philippines, Quezon City
Apolinaro D. Nazarea, Samuel Bernal

Monoclonal antibody based diagnostic test is under development for agaculture.

3.5.National Institutes of Biotechnology and Applied Microbiology, University of the Philippines, Los Banos
William G. Padolina

Several hybridomas were prepared to produce monoclonal antibodies to detect plant viruses. The Institutes have also equipment for semi-industrial scale monoclonal antibody production.

3.6.MEDTEST Inc.

Augusto L. Lingao

A genuine immunodiffusion test was developed and marketed for detection of alpha fetoprotein as a marker of hepatocarcinoma.

Remarks:

In the field of antibody based diagnostics one product (alpha fetoprotein test) is already commercialized, and it is very remarkable that it is an original product formulated to fit the conditions of rural areas of the Philippines.The other product which is near to the commercial stage is the Hepatitis B antigen kit.It is advisable to give support to this promising project for several reasons: (i) the product is very important for the country, because one part of blood donors are not screened for Hepatitis B, and (ii) it would give a boost to the development of the real Philippine biotechnology.

Other promising findings of this survey should be emphasized too:

i.In the National Institutes of Biotechnology & Applied Microbiology there are equipment and expertise for hybridoma preparation as well as small fermentors that can be used for monoclonal antibody production.

ii.Because of the concentration of most of the world's activity in biotechnology in developed countries the national strategy will require a variety of links to the industrialized world. There are numerous Philippino scientists and technologists working in universities and industrial establishments in the developed countries. Such persons represent a potentially very important channel for the Philippines to keep abreast of work relevant to the needs of the country. A promising project in this respect is the Molecular Biology and Biotechnology Program of the College of Science.

From industrial point of view I recomend to support two projects: production of blood typing sera and Hepatitis diagnostic kit.

Besides there are many important diagnostics that needs funding in the R&D stage:

(i) Schistosomiasis: Studies have been conducted in several countries on serodiagnostic tests for anti-S. mansoni and anti-S. japonicum antibodies. Several ELISA systems and an indium-slide assay have proved reliable for detection of schistosome infection. Crude schistosome egg preparations used as antigens in these assay systems provide sensitivity and specificity levels as good as the isolated egg antigens, and host serum levels of antibody to these crude egg antigens correlate well with schistosome egg output. These tests should be useful in the maintenance phase of disease control programes.
Tests have been develcped that are based on the detection of circulating parasite antigens or immune complexes and they are more specific for current infection. This tests have been improved by the use of monoclonal antibodies raised against schistosome antigens.

(ii) Malaria: During the past decade, several new diagnostic methods have been developed and monoclonal antibodies and cloned gen products are becoming available for use as standard reagents.
Monoclonal antibodies to circumsporozoite (CS) protein

inhibit the attachment of sporozoites to, and their penetration of, liver cells, suggesting that the epitope recognized by the antibody may be part of the molecule involved in the host-parasite interaction. On this assumption, an assay measuring the inhibition of sporozoite entry into cultured human hepatoma cells has been developed for the determination of protective antibodies.

Monoclonal antibodies to CS proteins also form the basis of a powerful new epidemiological tool, the Zavala test, a solid-phase immunoassay which detects sporozoites in infected mosquitos. It is now being used in several endemic areas to identify malaria vector species and measure intensity of transmission.

Considerable efforts have been made to develop immunological tests for large-scale screening of blood for malaria parasites. Assays using acquired antibodies obtained from the sera of immune adults have shown satisfactory levels of sensitivity. However, efforts to replace these non-standard antibodies with monoclonal antibodies have met with only limited success.

The most promising current approach to diagnosis of active infection involves highly sensitive, species-specific DNA probes.

4. REAGENTS FOR HEPATITIS B SURFACE ANTIGEN TESTING

In the Philippines Hepatitis B is highly endemic. Exposure rate averages 58% and Hepatitis B surface antigen (HBsAg) positivity is 12% (Abrigo et al.: Phil. J. Internal Medicine, 25:116-120, 1987). The infection may lead to chronic hepatitis, cirrhosis and primary hepatocellular carcinoma. To reduce the morbidity and mortality Hepatitis B infection should be controlled by screening and immunization.

For screening HBsAg is commonly used as a marker of Hepatitis B infection. Several methods were developed for testing. In the order of sensitivity the commonly used techniques are: immunodiffusion, counter electrophoresis, latex agglutination, reverse passive hemagglutination (RPHA), enzyme immunoassay (EIA) and radioimmunoassay (RIA). RPHA is less sensitive then EIA or RIA, but I am not aware of any case when Hepatitis B was transmitted by a RPHA negative - EIA or RIA positive specimen. RPHA is simple to use and in contrast to EIA or RIA no expensive equipment is needed (Fig.2).

In the Philippines HBsAg RPHA regent was developed as a

joint project of the Department of Health's Research Institute for Tropical Medicine and the University of Philippines' Liver Study Group, with the cooperation of the Philippine National Red Cross, and the support of the Japan International Cooperation Agency, the Philippine Council for Health Research and Development, and the World Health Organization. This product is over the R&D stage and ready for the commercial stage (See: Annex IV). If the industrial production starts, it will be the first sophisticated antibody based diagnostic product in the Philippines.

4.1.Needs for HBsAg testing in the Philippines

No data is available on the consumption or import of Hepatitis B diagnostics. It is not compulsory to screen the donor blood for HBsAg.

The Philippine National Red Cross (PNRC) has facilities for testing at the National Blood Center, at the 3 Regional Blood Centers and at the 34 Chapter Blood Centers. The 26 Blood Extension Services and the 4 Blood Stations collect and store blood but no test is done. At the Blood Centers all of the blood are screened for HBsAg,

and the number of donations is between 90,000 and 100,000 per annum. Recently the experimental product of the Research Institute for Tropical Medicine was used for HBsAg screening with satisfactory results. Presently the RPHA test of Green Cross (Japan) is used, and the PNRC is allowed to buy it for a low price - P/1000 per 100 tests [Source: Dr. C. O. Samson, Director, PNRC National Blood Program].

Besides the PNRC blood is collected in hospital blood banks and commercial blood banks. No consolidated annual data exists, and the guessed number is 200,000 as a total for the two sectors. Blood is not screened for HBsAg in the commercial blood banks. Some of the hospital blood banks (Makati Medical Center, Polymedic General Hospital, Philippine Heart Center, National Kidney Institute) test all the blood taken in the hospital or purchased from commercial blood banks. They use commercial RPHA tests (P/2900 per 100 tests) or EIA kits (P/5500 per 100 tests) for HBsAg screening. Some hospitals visited have no facilities for testing (Rizal Medical Center, Infant Jesus Hospital), and I was informed that it is not rare in the Philippines [see: The Supply of Source Materials for the Production of Human Serum Albumin and Gamma Globulin in the Philippines, DP/PHI/87/019]. Based on

this fragmentary data the actual consumption may be estimated to be between 200,000 and 300,000 tests per annum.

At the calculation of the actual needs the followings were assumed: (i) the Department of Health (DOH) makes the screening of donor blood obligatory for the blood banks and recomends the retesting in the hospitals, (ii)50 % of the government hospitals have facilities for screening, (iii) 75 % of private hospitals are performing HBsAg tests. They were assumed on the bases that (i) the DOH presently considering this steps (source: Undersecretary Dr. T. Maramba), and (ii and iii) the RPHA test needs no special expensive instrument, only laboratory space and a technician.

Immediate needs (1989)

	Samples/year
I. DOH/PNRC Requirements	
1. PNRC donor blood units	100,000
2. Retesting of blood in DOH hospitals 50% of 100,000	50,000
3. Screening of high risk groups 50% of 50,000	25,000

4. Patients in DOH hospitals/clinics	
See: Annex III	25,000
5. Research	5,000
Subtotal:	205,000

II. Private Sector Requirements

1. Non-PNRC donor blood units	200,000
2. Retesting of blood	
75% of 200,000	150,000
3. Screening of high risk groups	
75% of 150,000	112,000
4. Patients and relatives in private	
hospitals/clinics - see Annex III	72,000
5. Screening of pregnant women under	
private medical care - see Annex III	75,000
Subtotal:	659,000

Source: Liver Study Group, University of the Philippines, Manila

In general, 100 tests are sufficient for 60 determinations, because 40 tests are requested for control and rechecking the ambiguous results. Based on this data the calculated immediate need is 1,440,000 tests per annum.

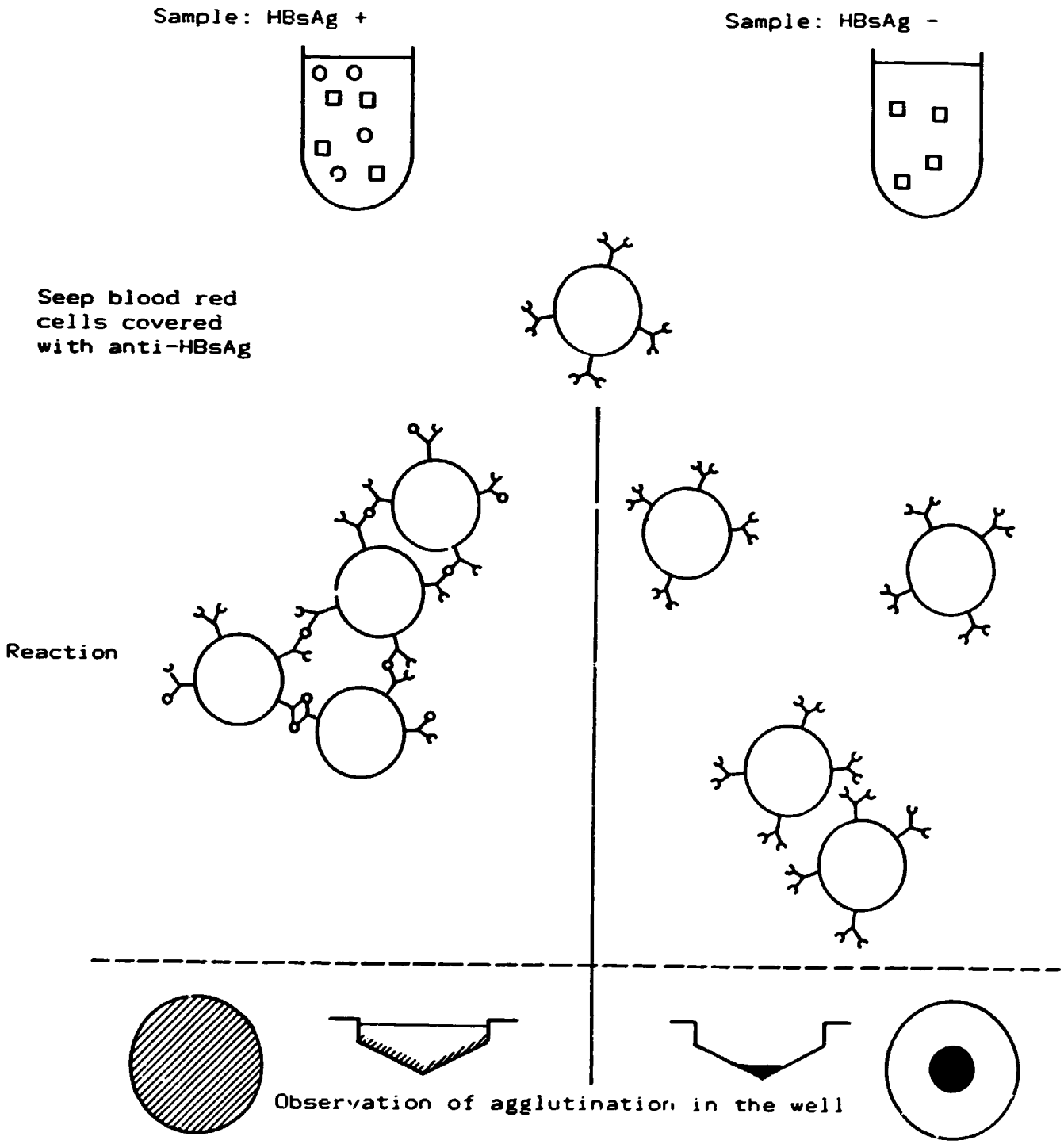

Fig. 2. Reverse passive hemagglutination assay
The reaction between antibodies attached to the surface of red cells and the antigen leads to hemagglutination.

4.2.Production of RPHA reagents for HBsAg testing

The main steps of the production are (Fig.3):

a.Isolation of HBsAg from positive plasma. Preparative ultracentrifuge is the basic equipment, and it costs $ 60,000. About 50% of the positive plasma contain HBsAg in sufficient quantity for separation. The yield is about 13 mg of purified preparate per liter plasma. Presently the positive plasma is free, but in the future the costs or blood banks should probably be covered, and it is roughly P/600 per liter.

b.Production of antibodies to HBsAg by immunization. An animal house for rabbits and a top desk centrifuge are the main requirements, and the investment would be about $ 50,000. 3 mg of purified HBsAg are needed for the immunization a rabbit and the yield is around 30 ml.

c.Preparation of immobilized HBsAg for immunoaffinity chromatography. Fume hood,pH meter,chromatographic columns and pumps are the basic equipment that may be purchased for $ 6000. 10 mg of purified HBsAg can be immobilized on

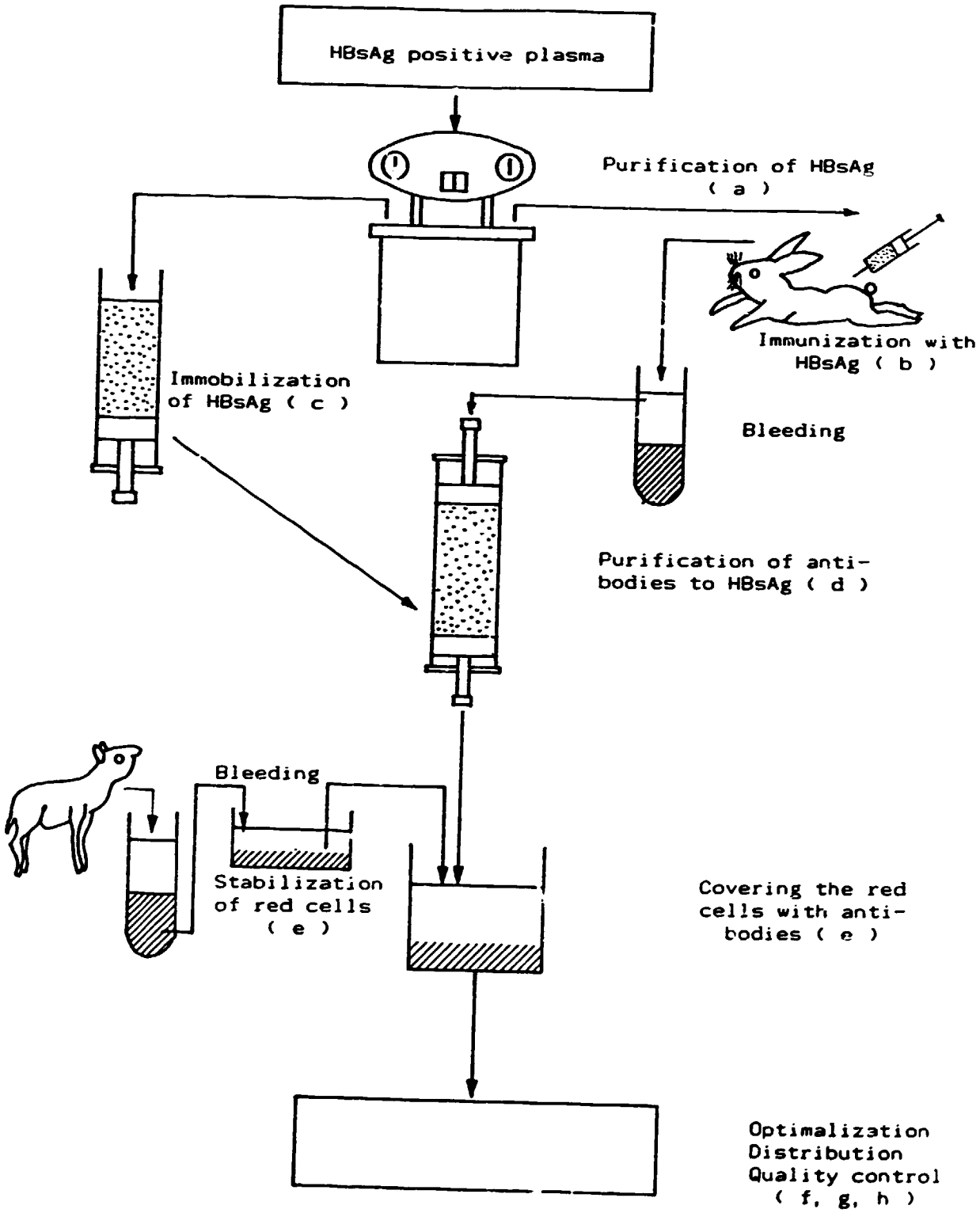

Fig. 3. Diagram of the procedure for production of HBsAg RPHA regent

one ml of gel, and it may be expected to loose 5 ml of immunoadsorbent in a purification cycle.

d.Purification of antibodies to HBsAg by immunoaffinity chromatography. Adsorbance monitor and fraction collector are the additional equipment and they can be purchased for $ 8000. It is possible to get 12 mg of antibody preparation from 100 ml of immune sera.

e.Preparation of antibody-coated sheep eryhrocytes. Animal house for sheep and incubator with shaker are needed at an approximate cost of $ 10,000. One mg of purified anti-HBsAg antibody is necessary to coat 20 ml of 5% stabilized sheep red blood cells.

f.Preparation of RPHA buffer and control sera. Sterile box, pressure holder filter with reservoir, air pump and pipetters are needed at a price of $ 6000. For 100,000 tests 20 l of RPHA buffer and 600 ml of control sera should be prepared.

g.Optimalization of the test system. No additional equipment is necessary.

h.Distribution of sensitized sheep red blood cells.

Quality control. No additional equipment is needed.

It can be calculated that for the production of 100,000 tests 18 litres of HBsAg positive plasma are needed as source material. 12 rabbits should be immunized and one sheep kept as a blood donor. 1500 vials of different sizes are required for packaging. The costs of fine chemicals and energy are not exceptional. Three technicians and one BSc could handle the work load for 300,000 tests per annum. The cost of production would be between P/1 and 2 per test.

The investment requirements are substatial ones. Besides the items mentioned in paragraphs a,b,c,d,e and f some other general laboratory instruments are also needed: balances, photometer, freezers, refrigerators, autoclave, sterilizers, etc. The total sum for equipment and animal houses is ruoghly $ 200,000 and additionally a laboratory area of 100 square metres is needed.

4.3. Institutional possibilities at the production and marketing

The Department of Health's Research Institute for Tropical

Medicine serves as a public health institution, as a center of R & D, but it is not supposed to be a production plant. It is advisable to transfer the manufacture to a specialized government laboratory or a private firm, and to use the scientists and technologists of the Research Institute to develop diagnostics for malaria or schistosomiasis.

A realistic price for the product would be about P/15 per test. In the field of antibody based diagnostics the wholesale price is usually ten times higher, than the actual cost of production to cover the costs of R & D and marketing. In case the screening of donor blood is compulsory for HBsAg, the selling of 300,000 tests per year can be expected. It means, that the value of the market for the domestic HBsAg RPHA kit is between P/4 and 5 millions. The consumption would probably grow by 10% yearly because of the high need.

A private firm should pay a fair price for the transfer of technology. The participants of the project (Research Institute for Tropical Medicine and the University of the Philippines' Liver Study Group) may sell the know how for a certain sum or/and for royalty payments.

The investment costs might be too high for a small firm, like MEDTEST, because of the taxes levied on imported items. In some countries, like Brazil, cuts up to 90% on import tariffs on equipment and 8% reductions in income tax would be granted to companies investing in biotechnology products. The burden of investment can be reduced by several arrangements: (i) transfer of technology step by step - first steps f and d, than e, etc, and the semi-processed products prepared in the Research Institute [steps a,b,etc] are bought by the manufacturer, (ii) leasing laboratory space, animal houses and equipment in the Reseach Institutein the first phase, and finance the investments from the income.

5. BLOOD TYPING REAGENTS

It is recomended to consider the production of the blood typing reagents in the Philippines for several reasons: (i) it may be profitable, (ii) it can reduce the burden of import, (iii) it helps to develop skills and expertise in the field of diagnostics and biotechnology.

5.1. Consumption of blood grouping reagents in the Philippines

Summarized customs data are available on the import of blood grouping reagents.

Year	Volume Net kg	FOB $
1982 *	9322	517,865
1983 *	7720	517,556
1984 *	7487	479,530
1985 *	9074	880,611
1986 *	8093	363,069
1987 **	10746	418,700

* Source: BOI, ** Source: Central Bank

It is difficult to evaluate the customs data , because the volume probably includes the weight of the whole kit.The main exporters are: USA, Germany, Netherlands and Australia.A lot of brands are competing for the market (Biotest, Biomedics, Organon, etc).

The present need can be estimated on the basis of blood donations, because generally five typing is made at one transfusion as an average. The estimated number of blood donations is 300,000 per annum (See report " The Supply of Source Materials for the Production of Human Serum Albumin and Gamma Globulin in the Philippines"DP/PHI/87/019),and it would require 1,500,000 typings in a year. This amount is expected to grow by 3-6 per cents per annum. The reagent requirements of the different test systems are different, but generally 100 tests need 6 ml of sera. It means that 90 litres are needed from the ABO and Rh typing sera. Calculating with prices of $1.2/ml for anti-A, $1/ml for anti-B, $1.1/ml for anti-AB and $1.6/ml for incomplete anti-D the sum is $441,000. It is not far from the customs data reported, and it means, that if the reagents are imported at world prices, the volume is sufficient to cover the need.

5.2.Production of blood typing reagents by traditional methods

It is advisable to develop the production in successive stages.

The first step may be the installation the facilities for sterile filtration, distribution, packaging and quality control. The cost of basic equipment for a capacity of 5-10 l/week [sterile box, pressure holder filter with reservoir, air pump, pipetters, refrigerator, freezer, autoclave] is about $ 25.000. The packaging materal can be produced locally. It is possible to purchase in bulk ABO typing sera for $ 0,2-0,5/ml in the world market. The distribution, packaging and quality control may be handled by three technicians. It is recomended to hire a well trained haematologist part time to supervise the quality control. It is estimated that the production at this stage may be profitable if more than 100 l of blood typing sera are marketed per annum, but a detailed feasibility study should be carried out.

The next stage is the local production of the reagents, and there are two possibilities: (i) to collect human sera

with good antibody titres or (ii) to produce monoclonal antibodies in cell cultures.

Blood typing reagents can be produced from sera that have antibody titre of 1/32 or higher. One possibility is the screening of donor blood for the presence of good antibody. This was tried at the Philippine National Red Cross (Dr.C. O. Samson) with moderate success. The blood donors are predominantly males in the Philippines, while the possibility of a good antibody titre is higher among females following deliveries. Additional drawbacks of this approach are the higher cost because the price of blood bags should be adsorbed , and the problem of plasma - serum conversion. An alternative possibility is the screening of blood obtained from undertakers.

The usual way of the blood typing sera production is the immunization of paid donors with antigens. Sterile and pirogen free blood group substances are available for $ 10-15 / vial. The fee for paid donors in the Philippines is about P/70, but a premium should be added for the immunization. Blood bags are not needed, the blood can be drawn directly to a sterile container. The standard blood bank equipment [refrigerated centrifuge, refrigerator, freezer, autoclave] is sufficient for the production of blood grouping sera, no additional investment is needed.

if it is prepared at a good hospital blood bank (see:Technical Report:The Supply of Source Materials for the Production of Human Serum Albumin and Gamma Globulin in the Philippines,DP/PHI/87/019).In case a commercial blood bank is contracted a medium quality centrifuge has to be purchased for about $ 6000. Technology and know how for immunisation and serum production can be obtained for $ 20.000-30.000, or through an international agency like UNIDO, or it can be developed by Philippine experts.

I think that the production, packaging, quality control and marketing can be organized effectively by a small specialized firm, like MEDTECH, while hospital blood banks or commercial blood banks are contracted for the actual immunization and blood collection. If the production starts with imported typing sera,they may be replaced step by step by locally produced materials. The cost of production can be estimated to be around P/4 per ml. An alternative solution is the establishment of a laboratory under the DOH as a non-profit organisation to provide reagents to the public health sector. The Philippine National Red Cross has less favorable possibilities, because only voluntary donors are used there.

5.3.Production of monoclonal antibodies for blood typing

Monoclonal antibodies can be prepared reproducibly in unlimited quantities at a moderate cost, so their use is growing. Some blood banks apply monoclonals in the Philippines too (Makati Medical Center). Hybridoma cell lines can be purchased for less than $ 50,000 and the conditions and expertise may be developed at the National Institutes of Biotechnology and Applied Microbiology for mass production of antibodies.

There are a number of important differences between microbial and mammalian cells which necessitate different approaches. In the case of microbial cells there is a considerable flexibility in the growth medium composition and tolerance to toxic metabolite accumulation. Growth of mammalian cells, on the other hand, is restricted to a narrow range of environmental conditions. Medium for mammalian cell culture consist of an osmotically balanced complex mixture of vitamins, minerals, amino acids. It is usually supplemented with fetal calf serum. The buffering system is analogous to circulating blood.The fragile nature of mammalian cells makes them sensitive to shear forces.

During the last years many new cell cultivation systems

have been developed. In general, these can be grouped into the following specifications: (i) homogeneous systems [including all suspension culture systems and microcarrier suspension cultures], and (ii) heterogeneous systems where the cells are immobilized, entrapped or encapsulated and therefore separated from the medium.

The classical system is the suspension culture using rotated bottles or stirred tank. The National Institutes of Biotechnology & Applied Microbiology have the basic equipment for hybridoma propagation. It is a challenging task to find out the proper medium, control and agitation systems, because they may be different from cell line to cell line. It is an art that needs imagination and intuition too, but I am confident that the Philippine scientists and technologists are capable to solve the problems.Suspension cultures can be propagated in batch, in fed batch and in chemostat systems. The fermentors they have can only be used batch-wise, but it is possible to alter them to fed batch system (Fig.4). Blood grouping antibody production is a good start in this field of biotechnology, because the down stream processing is simple: the cells have to be separated by centrifugation or/and filtration and the culture supernatant may be used as blood gouping reagent without purification (Fig.5). A.

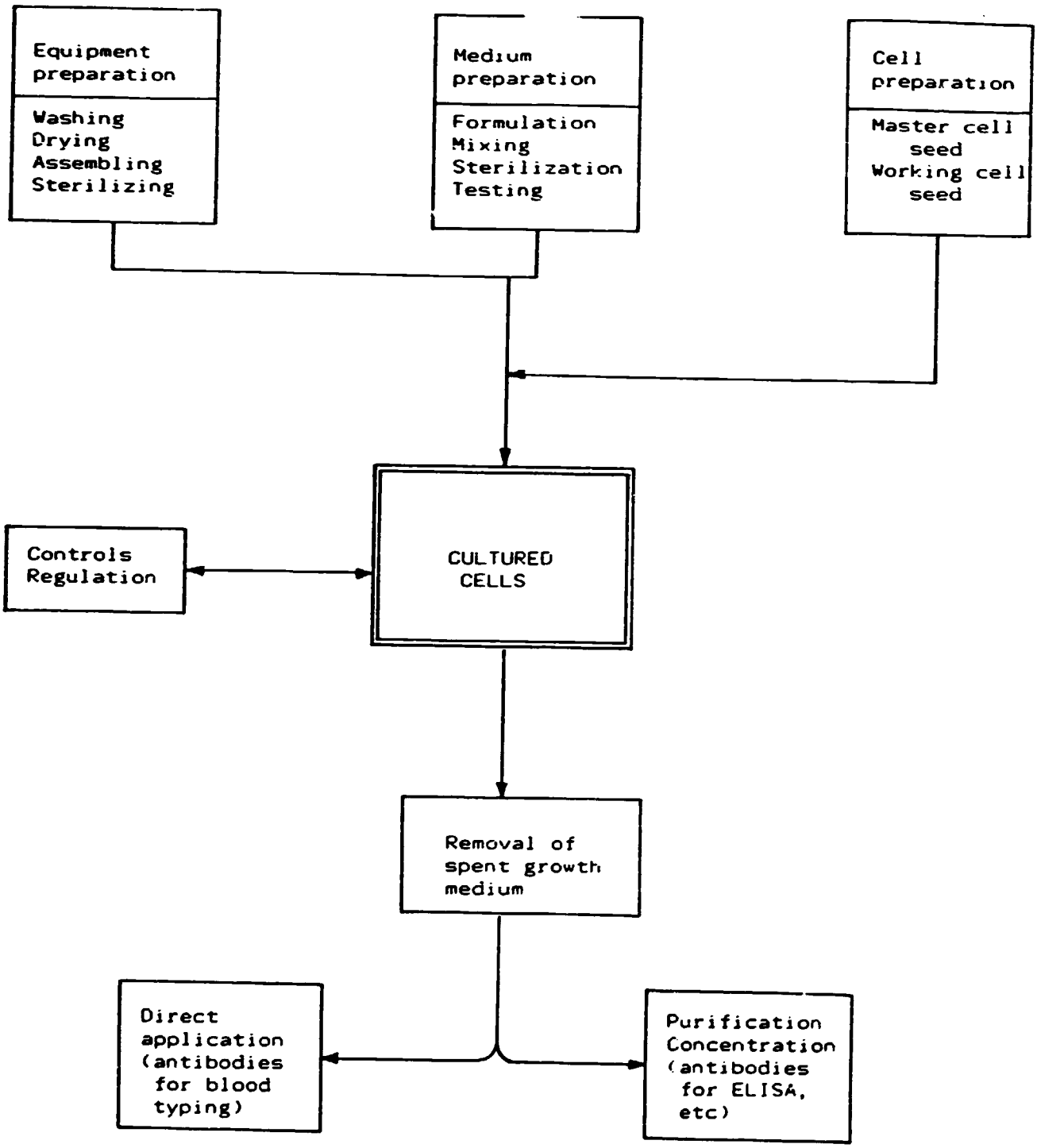

Fig. 4 Outline of the steps involved in generating monoclonal antibodies from hybridomas

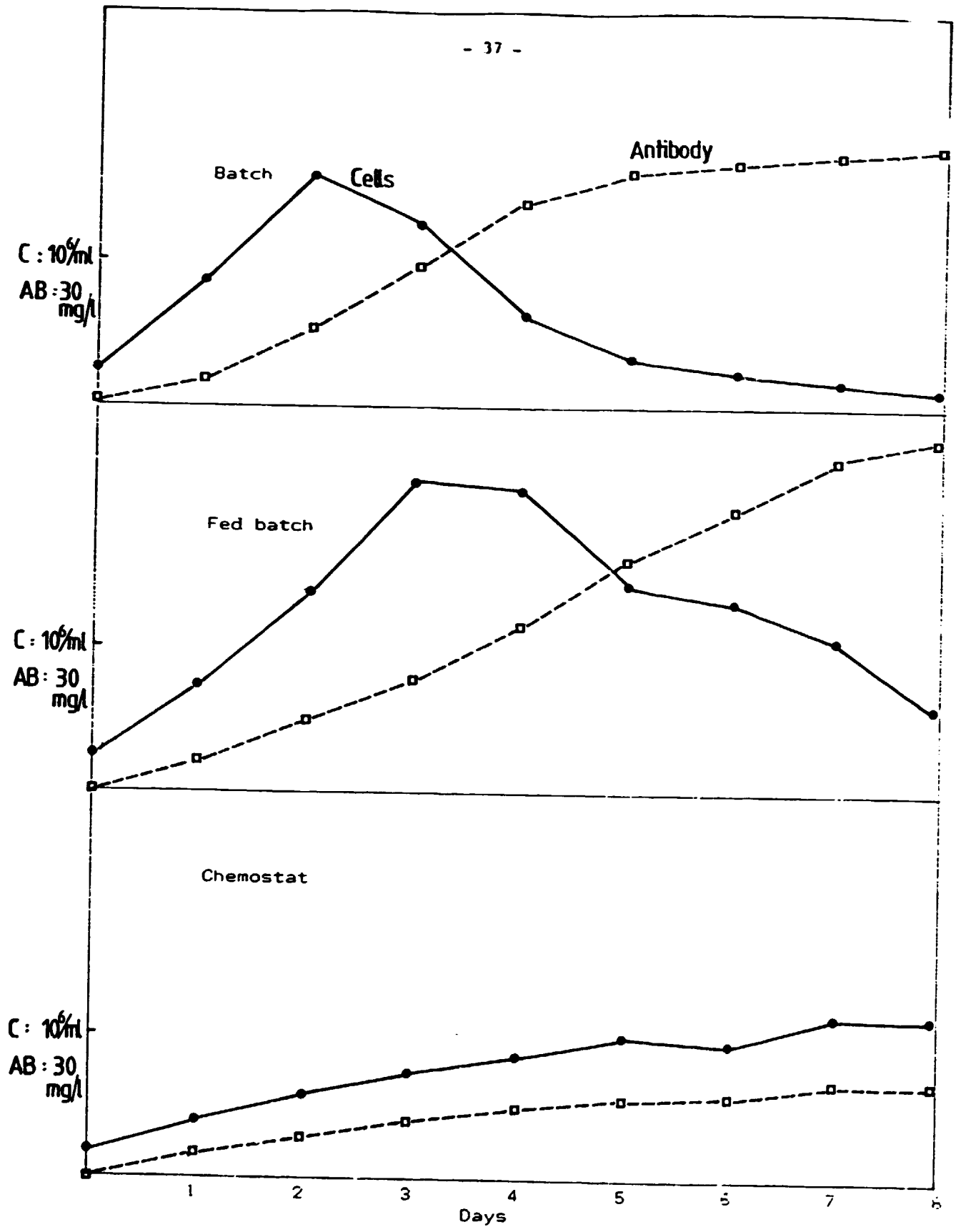

Fig. 5. Cell growth and monoclonal antibody production in batch, fed-batch and chemostat cultures

antibody titre of 1/32 is sufficient for the test, and it is possible to reach titres up to 1/256 with traditional systems too, and the growth media may be diluted accordingly. It is possible to cover the need of the country by 30 runs in a 10 litres fermentor.

It is impossible to avoid blind alleys and contaminations at the experimental stage, and for this reason only a rough estimation can be made on costs. A grant of $ 200.000 would probably cover the costs of additional equipment (roller bottle apparatuses may be fabricated in the Philippines), spare parts, media, reagents and salaries. A successful project would be beneficial for the development of modern biotechnology: the expertise acquired and the equipment can be used for the production of other monoclonals too. They are more and more important not only in the human and veterinary diagnosis but also in the fiels of plant diseases, toxins, environmental contaminants. One unit for mass production of monoclonal antibodies may serve several hybridoma producing laboratories in the country. An other positive development might be the enhancement of selfconfidence, independence and creativity of the Philippine scientists and technologists.

Fetal calf serum can be considered as a roadblock in the development. Its price is very high, because the demand is growing with the advance of biotechnology and in the supply side there is a shortage. The price of fetal calf serum was between P/10,000 and 20,000 per liter in the Philippines in 1988, and it is going up. One experimental run in a 10 l fermentor may require up to 1 liter of serum, and a project of modern biotechnology needs hundreds of litres. Domestic production should be considered to ensure the supply at a moderate price. It is possible to collect hundreds of litres of fetal like newborn calf serum in a dairy farm (300 ml/ calf) without harmful effects on cows and calfs [see: The Supply of Animal By-products for the Production of Bioactive Substances in the Philippines, DP/PHI/87/019]. The cost of equipment, furniture and technology is roughly $ 100.000 and one technician can manage the operation. It is possible to get serum for P/1200 per liter (purchase of blood, labour, operational costs included), if more then 300 litres are collected per annum. The sera may be screened for cell grown support properties , and the filtration, distribution and quality control can be performed at the National Institutes of Biotechnology & Applied Microbiology.

It might be possible to produce monoclonal blood typing reagents for about P/2 per ml, as a result of a successful biotechnological project. It is recomended for the National Institutes of Biotechnology & Applied Microbiology to sell it in bulk form to firms specialized in diagnostics.

ANNEX I

PHILIPPINES
DP/PHI/87/019
List of Persons Met

1.	Dr. Alfredo Bengzon	- Secretary of Health
2.	Mr. Rhais Gamboa	- Undersecretary of Health
3.	Dr. Quintin Kintanar	- Director PCHRD, Assistant Secretary of Health
4.	Dr. Leticia B. Gutierrez	- Professor U.P. College of Pharmacy
5.	Dr. Natividad de Castro	- University of the Philippines, College of Pharmacy
6.	Ms. Lydia M. Joson	- Chief, Microbiology and Genetics Division, Industrial Tech. Dev. Institute (ITDI)
7.	Mr. William Padolina	- Professor of Chemistry, U.P. Los Baños, Director of Biotech
8.	Dr. S. Quintana	- Prof. and Coordinator Medicinal Plants Project U.P. Los Baños
9.	Mr. Jose O. Juliano	- President and General Manager Interphil Laboratories
10.	Dr. Vicente X. Genato	- Vice-President Polymedic General Hospital
11.	Dr. Augusto L. Lingao	- President, Philippine Society of Allergology & Immunology
12.	Dr. Augusto Litonjua	- President Philippine Diabetes Association
13.	Dr. Amelia A. Garcia	- Department Head, Makati Medical Center
14.	Mr. Eduardo Cabrera	- Manufacturing Manager - Interphil Laboratories
15.	Dr. Alberto K. Alcantara	- President, Philippine Socity of Microbiology
16.	Capt. Larry Laughlin	- US Naval Medical Research Units NZ
17.	Dr. Thomas Maramba	- Undersecretary, DOH
18.	Dr. Manuel V. Cruz	- Professor, Department Obstetrics & Gynecology, UST
19.	Dr. Fortunato Sevilla	- Director, Research Center for Natural Science, UST
20.	Dr. Beatrice Guevara	- Research Center for Natural Science, UST
21.	Dr. Manuel Navarro	- Professor, Research Center for Natural Science, UST
22.	Ms. Criselda G. Abesamis	- Pathologist, Blood Coordinating Council
23.	Ms. Carmen T. Narciso	- Hematologist, Chief Blood Bank, Heart Center
24.	Mr. Celso O. Samson	- Director, Philippine Red Cross
25.	Dr. Ditas B. Javier	- Department Head, Rizal Medical Center
26.	Dr. Norma Ona	- Hematologist, Polymedic General Hospital
27.	Ms. Amelia Garcia	- Hematologist, Polymedic General Hospital
28.	Ms. Floricita C. Fernández	- R&D Superintendent, Magnolia Div.- Philippine Dairy Products
29.	Mr. Mario G. Cesorio	- Manufacturing Superintendent - Magnolia Division Philippine Dairy
30.	Ms. Elma G. Llaguno	- Department of Chemistry, UP
31.	Ms. Luisa S. Saniel	- Culture Collection & Institute of Biology NSRI
32.	Ms. Evangeline C. Santiago	- Analytical Services Laboratory NSRI
33.	Mr. Jorge A.K. Ochoa	- Culture Collection - Microbiology Unit NSRI
34.	Ms. Maria Auxillia Tan	- Culture Collection - Microbiology Unit NSRI
35.	Ms. Virginia S. Carino	- Institute of Biology, UP Diliman, QC

36.	Ms. Adoracion T. Aranez	- Institute of Biology, UP Diliman, QC
37.	Ms. Saturnina C. Halos	- NSRI, UP Diliman QC
38.	Dr. Edito G. Garcia	- Professor Inst. of Medical Parasitology UP Manila
39.	Dr. Amante G. Cruz	- Ass. Professor Ins. of Med. Parasitology UP Manila
40.	Dr. Nadia Marason	- Professor, Department of Microbiology UP Manila
41.	Dr. Antonio V. Jacalde	- Professor, Department of Microbiology UP Manila
42.	Ms. Luz Z. Lucas	- Antibiotics, Nat. Institute of Biotechnology
43.	Dr. Mediodora Saniel	- Director, Institute of Tropical Medicine
44.	Dr. Remigio Olveda	- Head, Institute of Tropical Medicine
45.	Dr. Rufino C. Liragi	- Director Industrial Technology Development Institute
46.	Mr. Antonio L. Gonzales	- Head, Department Organic Chemistry ITDI
47.	Ms. Dolores Isaac	- Head, Department Inorganic Chemistry ITDI
48.	Ms. Josefina B. Manalo	- Head, Department Pharmaceutical Chemistry, ITDI
49.	Ms. Qunlillano Montevile-gen	- Head, Chemical Process Development ITDI
50.	Dr. Esther Albano-Garcia	- Deputy Executive Director (PCASTRD)
51.	Ms. Mercedes Soriano	- Deputy Director, ITDI, (DOST)
52.	Dr. Ing. Adolfo Gopez	- Deputy Director, ITDI, (DOST)
53.	Dr. Eulalia Venzon	- Head Biology & Toxicology Dept. NIST (DOST)
54.	Dr. Veronica Chan	- Professor UP College of Medicine
55.	Dr. Ruben Aspiras	- Professor Microbial Physiology UPLB
56.	Dr. Asuncion Raymundo	- Professor Bacteriology UP, Los Baños
57.	Dr. Marita Reyes	- Professor UP. College of Medicine
58.	Dr. Hallen Molina	- UP Los Baños
59.	Dr. Ernesto del Rosario	- Professor of Chemistry UP, Los Baños
60.	Dr. Nelia Cortes-Maramba	- Professor of Pharmacology UP. College of Medicine
61.	Dr. Man Nancho	- Biologist Research Institute of Tropical Medicine
62.	Dr. Rufino Lirag	- Director ITDI, (DOST)
63.	Dr. L. Banez-Gutiérrez	- Professor UP College of Pharmacy
64.	Mr. Jose D. Pascual Jr.	- Operating Vice President, United Laboratories Inc.
65.	Ms. Estelita N. Garcia	- Assistant Vice President, United Laboratories Inc.
66.	Dr. Rogelio P. de Leon	- Director Analytical Chemistry Group. United Laboratories
67.	Dr. Benigno D. Peczon	- Analytical Research and Development, United Laboratories, Inc.
68.	Dr. A. D. Nazarea	- Programme Director, Molecular Biology and Biotechnology Program U.P. Diliman
69.	Dr. Samuel Bernal	- Ass. Professor, Division of Medicine Harvard Medical School
70.	Dr. Claro Santiago	- National Institute of Science and Technology
71.	Ms. Alicia G. Salazar	- Head, Antibiotic Section, Bureau of Food and Drugs
72.	Dr. Marlito L. Cardenas	- Head R&D, San Miguel Corporation

73.	Dr. Carlito R. Barril	- Professor, Institute of Chemistry, UPLB
74.	Dr. Lydia C. Crisostomo	- Chief, Laboratory Services Division Bureau of Plant Industry
75.	Dr. Augusto G. Santos-Ocampo	- Chairman, Board of Chemistry, Professional Regulatory Commission

Annex II

List of Priorities

Medical

Priority I

1. Vaccines
2. Drugs (Antibiotics)
   - drugs that have local raw/intermediate materials
   - fermentation; chemical synthesis; extraction and purification from natural products

   for the ff. DOH priority diseases:
   1. TB
   2. Malaria
   3. Schistosomiasis
   4. Leprosy
   5. Diarrhea
   6. ARI
3. Diagnostic Agents

Priority II

4. Nutritional Supplements/vitamins

Priority III

5. Toxicology (human)

Source: Department of Science and Technology

Annex III Needs for HBsAg testing

25,000 HBsAg tests for patients in DOH hospitals (within next 2 years)

Premises:

1. Philippine population	= 55,000,000
2. 80% lower socio-economic levels (LSEL) and dependent on government health care system	= 44,000,000
3. 12% of average HBsAg positivity of LSEL	= 5,280,000
4. 10% of HBsAg (+) will be symptomatic	= 528,000
5. 50% of #4 will avail of government health care delivery system	= 250,000
6. 10% of #5 will be tested for HBsAg, considering logistics, HBV consciousness of government health workers, etc.	= 25,000

HBsAg tests for private patients in next 2 years

Premises:

1. Philippine population	= 55,000,000
2. 20% from middle and upper socio-economic level and under private health care	= 11,000,000
3. 3% HBsAg positivity of M-USEL	= 330,000
4. 10% of #3 will seek medical care because of of symptoms	= 30,000
5. 80% of no.4 will undergo tests	= 24,000
6. Average of 2 family members/relatives of #5 will undergo tests	= 48,000

HBsAg screening of pregnant women under private medical care

Premises:

1. 1.5 million births/yr	
2. 10% of #1 from private sector	= 150,000/year
3. 50% consciousness, logistics, etc.	= 75,000/year

Source: Liver Study Group, University of the Philippines

HBsAg RPHA REAGENT
(RITM - UP-LSG)

Description: Reverse passive hemagglutination test for the detection of HBsAg.

Principle: HBsAg RPHA Reagent (RITM-UP-LSG) is a test based on the principle of reverse passive hemagglutination (RPHA) in which stabilized sheep red cells previously sensitized with rabbit monospecific antibody to HBsAg, agglutinate in the presence of HBsAg in the serum.

Materials Provided:

1. One vial of 2 ml. 8% sheep red blood cells sensitized with monospecific antiHBs.
2. Two vials of 35 ml. RPHA buffer.
3. One vial of 1 ml. HBsAg (+) control serum ($2^{4.5}$).
4. One vial of 1 ml. HBsAg (-) control serum.

Procedure and Interpretation of Qualitative Screening Test Using Microtitration Technique:

Materials Needed:

1. Microplate (V shape) 10 x 12 wells
2. Microdiluters (0.025 ml. test capacity)
3. Dropper (0.025 ml)
4. Mixer (Optional)
5. Reading Mirror (Optional)

Preparation of Working RPHA Reagent:

RPHA cell (8%, 2 ml.) - decant and under aseptic technique, add 20 ml. RPHA buffer to make 0.8% suspension.

H B s A g R P H A R E A G E N T

(RITM - UP-LSG)

This HBsAg RPHA Reagent was produced as a joint project of the Department of Health's Research Institute for Tropical Medicine and the University of the Philippines' Liver Study Group, with the cooperation of the Philippine National Red Cross, and the support of the Japan International Cooperation Agency, the Philippine Council for Health Research and Development, and the World Health Organization.

Test Procedure:

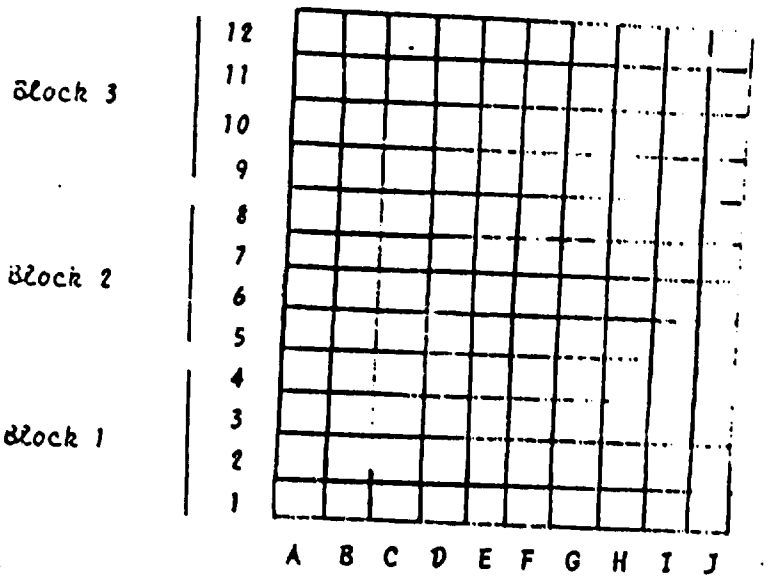

Fig. 1

1. Divide a 10 x 12 well plate into 3 blocks.
2. Place 1 drop (0.025 ml.) of RPHA buffer to each well.
3. Place 0.025 ml. of the test sample with a diluter in the first wells of each block (A1, B1, . . . J1; A5, B5, . . . J5; A9, B9, . . . J9), and make serial 2-fold dilutions to produce 4 dilution levels, i.e., 1:2, 1:4, 1:8, & 1:16.
4. Always include a (+) and a (-) control in each plate.
5. Add 1 drop (.025 ml.) of reconstituted (.8%) RPHA cells to wells 3 & 4 of block 1, wells 7 & 8 of block 2 and wells 11 & 12 of block 3.
6. Shake in a mixer, or tap 10 times using the side of your palm.
7. Incubate for 1-2 hours at room temperature on a flat table. Make sure that there is no vibration and any other movement of the plate during incubation.
8. Read hemagglutination titer as follows:

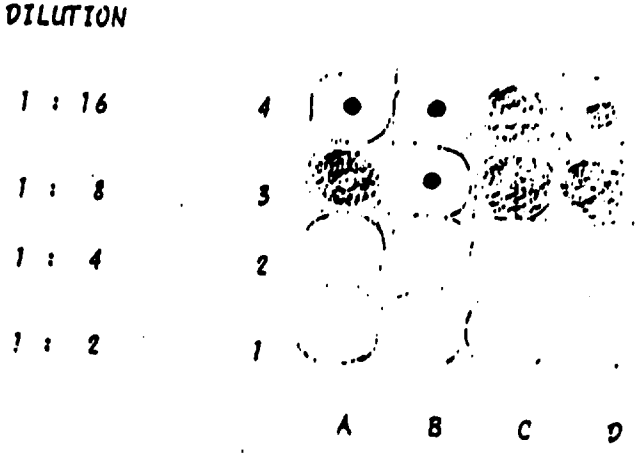

Fig. 2

SPECIMEN	RESULT	TITER
A	+	2^3 = 1 : 8
B	-	
C	+	2^4 = 1 : 16
D	+	$2^{3.5}$ = 1 : 12

Table I

9. Serum specimens showing agglutination at wells 3 and/or 4 are considered screening positive with this RPHA technic (Table I).
10. It is advisable to confirm the results of sera which are positive in the screening test by using inhibition tests or other techniques such as radioimmunoassay or enzyme immunoassay.

Precautions:

1. The RPHA cell stock (8%) and working suspension (0.8%) should be stored at 2 - 8°C. Do not freeze.
2. Avoid contamination of the working solution.
3. Always use the RPHA buffer dropper (.025 ml.) only for the RPHA buffer.
4. Always use the RPHA cell dropper (.025 ml.) only for the RPHA cell reagent.*
5. Blot dry on tissue paper all clean microdiluters before use.
6. Clean the microtiter plates as follows:
   a) Wash plates in running water and soak in a solution of liquid detergent and water overnight
   b) Next day, rinse plates by dipping into a basin full of water and shake away the trapped water 12x and repeat procedure using distilled H_2O this time (5x). Note: Make sure that cell buttons are removed.
   c) Dry in drying shelf face down.
7. Clean the microdiluters as follows:
   a) After each use, place microdiluters in a glass container with a little amount of water. Label the glass "DIRTY".
   b) Wash microdiluters by rotating under running water for 10 times, changing the water inside the glass each time.
   c) Repeat (b) using distilled water (3x).
   d) Place microdiluters in a glass container with a little amount of distilled water. Label the container "CLEAN".

   Note: Flame microdiluters once a week.
8. All reagents and serum samples should be handled as potentially infectious.

*Shake gently the RPHA cell reagent before use.

Storage: 2 - 8°C. Do not freeze.

Shelf life:

1. Stock Reagents: 1 year from production date. Refer to vial label for expiration date.
2. Working Reagents: 4 months after reconstitution.

Maximum assays with 2 ml. vial of stock RPHA Cell Reagent: 400 x 2-point screening tests