Development Leading to the Production of Immunological Reagents in Thailand*

Bencha Petchclai, M.D.
Kulayanee Khupulsup, M.Sc.

The clinical Immunology Laboratory of Ramathibodi Hospital, which was established in 1968, gradually has become a leader in its field. It performs a large variety of high quality immunological tests at low cost for Ramathibodi Hospital and all the other hospitals in the surrounding area of Thailand's capital. Beside its function as an unofficial central clinical immunology laboratory, it plays a major role in supplying immunological reagents to hospitals throughout Thailand. Soon experts from this laboratory will advise a national laboratory in the region about setting up a similar unit. A small laboratory is, nonetheless leading Thailand towards self-reliance in the production of immunological reagents.

The following information on the development process will provide some insight to its activities and may act as a guide for other laboratories to follow.

DEVELOPMENT PROCESS

Problems stemming from reliance on imported immunological reagents were the main driving force behind the effort to begin the production locally of these reagents. The price of the imported products generally were too high for the local economy and the supply was sometimes unreliable, especially for rare reagents. Also, the packages of some imported kits were too large for average hospitals in Thailand to use effectively and some techniques were unsuitable for the equipment and personnel locally available. Many reagents reached the users just shortly before the expiration date, and some even went past the expiration date during transit. These were among the problems that eventually forced many to realise that an improvement in laboratory diagnosis, aimed at providing better health care in developing countries, could not remain dependent on imported immunological reagents.

We arrived at the same conclusion and decided on our own to produce immunological reagents that enjoy a large demand; an immunological pregnancy test was the first reagent developed in 1969.

For several years, our laboratory continued to develop more immunological reagents at a slow pace without definite direction. Then we reached a turning point. Mahidol University together with the World Health Organisation decided to support the local production of immunological reagents. Two regional workshops for the purpose were conducted at our laboratory to stimulate interest, then the necessary equipment for local production of immunological reagents was provided by W.H.O. and suitable personnel were sent to the Department of Immunology, University of Birmingham, England, for a short training course in the production of antisera. From then on the production of immunological reagents in Thailand has made good progress.

We approached the problem step by step. First we identified the need for immunological reagents. As the most active clinical immunology laboratory, we knew what this country needed and which test(s) we should develop and then we set priorities in light of what was possible. The right person to develop each test was identified. Some tests were developed by our staff and others by graduate students. Once a test reagent is developed, it is subjected to trial in our laboratory, then distributed to the provincial hospitals for field trials to determine whether it is suitable for use in the rural areas. The tests distributed also enable clinicians to familiarise themselves with the new test. Finally, proper packages and labels are made, and the reagents released for general use.

Although there are many immunological techniques, we have select-

*From the Clinical Immunology Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Bangkok 10400, Thailand.

ed just a few for development. The techniques must be specific, sensitive, simple, stable under storage and inexpensive. If possible,tests should be rapid. Based on the aforementioned requirements, indirect haemagglutination (IHA), reverse passive haemagglutination (RPHA), haemagglutination inhibition (HI), latex agglutination (LA) and immunodiffusion (ID) are the techniques employed by our laboratory. These techniques are most suited to the facilities and personnel available in rural areas. We develop some antisera to human serum proteins for single radial immunodiffusion (SRID), and reagents for enzyme-linked immunosorbent assay (ELISA), but these techniques an rarely used in the provinces. The list of tests available from our laboratory are shown in the table to the right. The following reagent kits are being distributed on a nation-wide scale: HI test for morphine in urine, IHA test for antibody to *Entamoeba histolytica*, RPHA test for alpha foeto-protein (AFP), RPHA test for hepatitis B surface antigen (HBsAg), anti DNA by IHA. These reagents solved the problems involved with imported reagents in the following respects: (1) All are used for laboratory diagnosis of diseases common to Thailand. The local needs for tests for HBsAg, AFP and antibody to *E. histolytica* are 1,000,000; 100,000 and 20,000 tests per year respectively. (2) All reagents are always available. (3) All can be used at every hospital in Thailand without additional equipment. (4) Tests for AFP and antibody to *E. histolytica* are stable in solution at 4°C which makes it possible to keep unused reagents. Equivalent imported reagents are available in lyophilised form which have to be used quickly after reconstitution. (5) Most reagents permit semiquantification, while equivalent imported reagents are qualitative. (6) The reagents are made available at prices 1/4 to 1/10 those of imported ones, thus all hospitals in Thailand can afford the tests.

Table 1 Immunological reagents produced at the Clinical Immunology Laboratory

Reagent	Technique
1. Ab to *Aspergillus*	ID
2. Ab to *B. pertussis*	Agglutination
3. Ab to diphtheria toxin	IHA
4. Ab to *Entamoeba histolytica*	IHA
5. Anti HB	PHA, ELISA
6. Ab to *Histoplasma*	ID
7. Ab to *Leptospira*	IHA
8. Ab to *Legionella pneumophila*	MA
9. Ab to *Mycoplasma pneumoniae*	IHA
10. Ab to *Pseudomonas pseudomallei*	IHA
11. Ab to *Salmonella*	IHA
12. Ab to *Streptococcus* group A exo-enzymes	IHA
13. Ab to *Taenia solium* in CSF	IHA
14. Ab to tetanus toxin	IHA
15. Ab to *Toxoplasma*	IHA
16. Ag of *H. influenzae* type B in CSF	LA, RPHA
17. Ag of *Meningococcus* in CSF	LA, RPHA
18. Ag of *Pneumococcus* in CSF	LA, RPHA
19. Rotavirus in stool	RPHA
20. Heat labile toxin (LT) of *Escherichia coli*	SCA
21. Anti DNA	IHA
22. Anti thyroglobulin	IHA
23. Rheumatoid factor	IHA, LA
24. Pregnancy test (quantitative)	HI
25. Fibrin degradation products	RPHA
26. Alpha foeto-protein	RPHA
27. Morphine in urine	HI
28. Myoglobin in urine	HI
29. Serum C 3	SRID
30. Ag of *Cryptococcus neoformans* in CSF	LA

ID = immunodiffusion
MA = micro-agglutination
IHA = indiect haemagglutination
HI = haemagglutination inhibition
RPHA = reverse passive haemagglutination
PHA = passive haemagglutination
LA = latex agglutination
ELISA = enzyme-linked immunosorbent assay
SRID = single radial immunodiffusion
SCA = staphylococcal coagglutination

The impact of our effort is felt throughout the country. We are responsible for the introduction of the tests that we invented, many of which were practically unknown to average clinicians in the rural areas. Screening of blood donors for HBsAg by RPHA is facilitated by our reagent. We were given an award for our HI test for morphine by the National Research Council which considered it as a useful invention for the country. It is hoped that more reagents which are now in routine use in the laboratory (see table above) will be released to the public.

FUTURE PLAN

There are many aspects concerning immunodiagnostic reagents that need further development. These aspacts require access to raw materials which are the products of new technologies, which include monoclonal antibodies, DNA pro-

bes and other materials derived from genetic engineering. We have reached a stage where sophisticated technologies are needed to produce simple immunological reagents for use in a developing country such as ours. These raw materials must be purchased whenever possible. Local production of the raw materials must be undertaken when necessary through national and international collaboration. Lacking such an approach, the general population in developing countries will be deprived of the needed tools for diagnosing highly prevalent diseases affecting them.

REMARKS AND CONCLUSION

The personnel in our laboratory comprising one M.D., one M.Sc., and five medical technologists devoted only part of their time to the project. We demonstrated that a lot can be accomplished when support and incentive are available to a small group of local talents who work in a suitable environment. If all the available resources of developing countries can be utilised for such a purpose, self-reliance in the production of immunological reagents would be a reality.