

THE IMMUNOASSAY SERVICES IN MALAYSIA

SL CH'NG MBBS, MRCPath*

INTRODUCTION

The current methods used in routine chemical pathological laboratory such as colorimetry, fluorimetry and absorption flame photometry are insensitive to biological substances at 10^{-11} to 10^{-17} mole range. The search for a sensitive and specific technique culminated in the development of saturation analysis using radioactive labels (Radioimmunoassay) by Yalow & Berson in 1960 for insulin assay¹, Ekins in 1960² for thyroxine assay and Barakat in 1961³ for Vitamin B₁₂ assay. Subsequent improvement in the techniques of protein isolation and iodination⁴, antibody production and antigen antibody complex separation⁵ led to considerable refinement of the methods which enable large scale production of reagents and commercial kits.

This sensitive and relatively specific radioimmunoassay (RIA) technique promoted rapid development in endocrinology and related fields. The widespread availability of kits also enabled an instant transfer of RIA methodology to clinical laboratories in developing countries which lack supportive biotechnology for the development of in-house methods. The drawbacks of radioimmunoassay include radiation hazard, the use and disposal of radioisotope labels, chemical instability of the labels and availability of only few radioisotopes for practical use. This prompted further search and development of alternative labels⁶ such as enzyme labels and fluorescence labels which had been applied successfully to several routine assays such as thyroxine, cortisol, human chorionic gonadotrophin, ferritin, phenytoin, phenobarbitone and morphine. The antigen antibody reaction can be monitored by colorimetry, spectrophotometry and fluorimetry available in most chemical pathological laboratories in the developing countries.

CURRENT STATUS

The immunoassay techniques were introduced initially into this country for research purpose, and was subsequently improved and modified for routine services. Vitamin B₁₂ assay using ⁵⁷Cobalt was first set up in 1969 for nutritional anaemia research in invitro

radioisotope laboratory of University Hospital, and it quickly became routine service. In 1977, following the establishment of the division of Radiochemistry, the Institute for Medical Research (IMR) involved actively with the World Health Organisation (W.H.O.) in human infertility and reproductive research. This prompted the introduction of hormonal assays such as human placental lactogen (HPL), follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol-17B, progesterone, testosterone and prolactin^{7,8}. These hormonal assays were subsequently offered on a routine basis to other laboratories in the country.

In the second phase of development of immunoassay services, one sees the direct application of immunoassay techniques to routine services rather than for research use. In endocrinology, the immunoassay technique was found to be far more superior to the *in vivo* radioisotopic methodology or chemical technique. This led to the introduction of thyroxine (T₄) triiodothyronine uptake test (T₃ uptake), triiodothyronine (T₃), thyroid stimulating hormone (TSH) and cortisol for routine services in the University Hospital and I.M.R. In the late seventies, the clinical awareness of the important concept of precise and sensitive therapeutic monitoring of neoplastic diseases of local importance such as trophoblastic tumour and hepatocarcinoma had prompted the set up of human chorionic gonadotrophin (HCG) and alphafetoprotein (AFP) assays. The importance of ferritin assay for the monitoring of chelation therapy in thalassaemia is gradually being realised. Most of these assays are available in the immunoassay laboratories of University Hospital and I.M.R. (see Table 1).

In toxicology the need of a rapid and sensitive screening test for the detection of opiate groups of drugs in drug addicts encouraged the introduction of opiate screening by immunoassay technique (mainly non isotopic) into the country. The clinical demand for accurate therapeutic drug monitoring further influenced the set up of drug assays using homogenous enzyme immunoassay system for phenobarbitone, phenytoin and

*Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur (Address for reprint requests).

digoxin. The high cost of the assay limits the range of tests available. These assays are offered as routine services in the Chemical Pathology Laboratories of General Hospital and University Hospital of Kuala Lumpur.

In Microbiology, few attempts have been made to apply immunoassay techniques to the detection of communicable diseases such as tuberculosis and malaria in rural areas. Screening of blood donor for Hepatitis B Surface Antigen, using immunoassay kits was introduced in late seventies in the blood bank of General Hospital and University Hospital of Kuala Lumpur because of the high local carrier rate for the virus. The high cost of screening prohibits the initiation of local population screening programmes using immunoassay techniques for treatable inborn metabolic diseases such as congenital hypothyroidism.

A fairly comprehensive immunoassay service is now being developed in the Chemical Pathology Laboratories of University Kebangsaan and the new medical school of University Sains Malaysia.

Specimen referral is practiced regularly or occasionally between the central laboratories. Most central laboratories do not have in-house methodology and have to rely on commercial radioimmunoassay kits or supplies of reagents by the World Health Organisation (W.H.O.) through research programmes. These laboratories participate to some extent in external quality assessment programme of commercial or non-commercial schemes provided by Wellcome, W.H.O. and I.A.E.A. (International Atomic Energy Agency). Internal quality control of assay performance is practised regularly by most laboratories, using pooled sera or commercial sera with assigned values. In recent years several central laboratories are participating in the I.A.E.A. internal quality control programme using a handheld programmable calculator which is also suitable for use in the peripheral laboratories. Internal monitoring of the gamma scintillation counter drift using ^{129}I Iodine had also been implemented in some laboratories. At the present moment there is no coordinated national scheme of external quality assessment for immunoassay in the central and peripheral laboratories.

In the main teaching hospitals, requests for infrequently performed tests such as adrenocorticotrophic hormone (ACTH) and parathormone (PTH) are referred directly by the clinician concerned to research or diagnostic laboratories abroad. There is no national system of referral for these rarely requested

assays.

The immunoassay services of clinical laboratories in General Hospitals in Kuala Lumpur, West and East Malaysia focus mainly on T_4 and opiate screening using the homogenous enzyme immunoassay technique (see table II). These tests are carried out only in pathology laboratories of the State General Hospitals. There are no separate divisions of radioisotope laboratories in these hospitals. Requests for other hormone assays are referred mainly to I.M.R. The teaching hospitals do accept occasional specimens from peripheral laboratories. The private laboratories also carry out some immunoassay for T_4 and make use of the main central laboratories for other hormone assays.

PROBLEMS OF PERIPHERAL LABORATORIES

The problems of the immunoassay services in the state general hospital laboratories are different from those of the main teaching and research centres. The aspiration of the manager of the peripheral clinical laboratory is to provide routine hormone assay services for commonly requested tests such as T_4 , T_3 , TSH, Cortisol, HCG and others of local therapeutic importance without sample referral to the central laboratories. This requires considerable financial commitment for the purchase of expensive gamma scintillation counters, refrigerated centrifuges and reagents such as kits. The laboratory manager also faces problems related to the shelf lives of the radioactive tracers, quality assessment and control of the assays and the disposal of radioactive wastes. There is a need for a simple, robust and cheap methodology for these commonly requested assays.

PROBLEMS OF THE CENTRAL LABORATORIES

The teaching hospital and IMR laboratories face different type of problems. The annual increase in workload is responsible for the yearly budgetary increase. Figure 1 shows that introduction of new tests in the radioisotope laboratory is followed by a phase of rapid increase in workload which reaches a plateau by three years. This probably represents the maximal number of logical clinical requests for a given hospital population which fails to show a similar trend of increase. A similar pattern of change is noted in the workload of I.M.R. as shown in figure 2. The rise in workload can be alleviated by devolution of some of the existing assays to the peripheral

laboratories supported by sensible clinical use of the tests which would further improve the pick-up rate of the assay⁹. Future introduction of any new test should always be supported by extra budgetary provision for the expected annual increase in the workload before the plateau stage.

In the developed countries, new sophisticated tests are introduced yearly in the clinical laboratories. The central laboratories of the developing countries are under constant pressure to follow suit to provide a comprehensive range of hormonal assays including the infrequently requested tests such as antidiuretic hormone, gastrointestinal polypeptides and neuropeptides which are of more academic than diagnostic importance and serve mainly to improve the status of the hospitals concerned. At the present stage of development no laboratory in this region is equipped to offer such a wide range of services. Referral of specimens to laboratories abroad is the answer at the present moment. Improved techniques in the preservation and transportation of specimens would help to reduce the cost of shipment. To establish comprehensive assay services locally is the ultimate goal of the immunoassayist in this country. This can only be realised at the central laboratories backed up by concurrent development in biotechnology and related facilities.

The practice of quality assessment and control of assay performance in the central and peripheral laboratories is far from satisfactory. There is no coordinated national quality control scheme for radioimmunoassay. The commercial Wellcome Immunoassay Quality Control Programme is expensive whereas non commercial programmes have a limited life span and eventually have to be replaced by a national quality control scheme.

FUTURE DEVELOPMENT

The health authorities should consider seriously the establishment of a central facility for purification of enzymes, hormones for preparation of antisera, standards and labelling of antigen with the aim of supplying reagents at low cost to all the central and peripheral laboratories. The centres concerned should carry out methodological research to improve assay design and to develop new techniques especially the non-isotopic immunoassay systems. The reagents for the latter have longer shelf lives, are cheaper to manufacture and free from radioactive hazard when compared to radioimmunoassay techniques. The assay can be performed in laboratories

equipped with colorimeters or spectrophotometers which are available in most peripheral laboratories. This would definitely help to speed up the decentralisation of tests to the peripheral laboratories. Attention should also be focussed on the application of immunoassay techniques for the detection of communicable diseases e.g. tuberculosis and malaria in the rural areas and screening of treatable inborn metabolic diseases such as congenital hypothyroidism.

A professional body under the auspices of Malaysian Society of Pathologists should be established. Its function would be to provide immunoassay training for chemical pathologists, clinical biochemists and technologists and to offer expert advice on matters such as quality control, evaluation and monitoring of kits, and in association with PUSPATI,* to draw up a detailed code of practice for the use of radioisotopes in the laboratories. The professional body should also be involved actively in the setting up of a national quality assessment scheme for the laboratories in teaching hospitals, government hospitals and private sectors.

At the state general hospital level, provision of immunoassay services as part of laboratory diagnostic services should be implemented. The chemical pathologists with an interest in immunoassay techniques would be responsible for providing a consultative service and for setting up of assays of local therapeutic importance such as thyroid stimulating hormone, cortisol, human chorionic gonadotrophin and alphafetoprotein. With the assistance of the clinical biochemist, he is also responsible for the quality of the assay and for implementation of devolution of tests from the central laboratories. The central laboratories should work together to offer new assays such as adrenocorticotrophic hormone, insulin, parathormone, renin and aldosterone to all the laboratories in the country. With the improvement in the quality and standard of laboratory medicine, these tests will gradually be decentralised to the peripheral laboratories.

CONCLUSION

The development of the radioimmunoassay services should be coordinated closely with the overall development in health care. The limiting factors are financial constraint, availability of the trained staff and the concurrent development of biotechnology. In Malaysia, the immunoassay service is in its infancy and

*Pusat Penyelidikan Atom Tun Ismail.

successful development in this field will improve diagnostic services to the patient and facilitate further research into the disease process in Malaysians.

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REFERENCES

1. Yalow RS and Barson SA. Immunoassay of endogenous plasma insulin in man J Clin Invest 1960; 39: 1157-75.
2. Ekin RP. The estimation of thyroxine in human plasma by an electrophoresis technique. Clin Chem Acta 1960; 5:453-59.
3. Barakat RM and Ekin RP. Assay of vitamin

- B., in blood. Lancet 1961; 2:25-26.
4. Greenwood FC, Hunter WM and Glover JS. The preparation of ¹³¹I labelled human growth hormone of high specific radioactivity Biochem J 1963; 89:114-23.
5. Ratcliffe JG. Separation technique in saturation analysis. Br Med Bull 1974; 30:1:32-37.
6. Roy F Schall Jr and Hal J Tenoso. Alternative to radioimmunoassay; labels and methods. Clin Chem 1981; 27:7:1157-64.
7. Annual Report 1977, Institute for Medical Research, Kuala Lumpur, Malaysia.
8. Annual Report 1981, Institute for Medical Research, Kuala Lumpur, Malaysia.
9. Ch'ng SL, Peh SC, Low EC, et al. The critical evaluation of the medical usefulness of thyroid function tests. 2nd Asian Pacific Congress of Clinical Biochemistry, 1982, Singapore.

TABLE 1
IMMUNOASSAY TESTS

	Available at the Invitro Radioisotope Laboratory University Hospital	Available at the Radiochemistry division of I.M.R.
Thyroxine	t	t
Triiodothyronine uptake test	+	-
Triiodothyronine	+	t
Thyroid-stimulating hormone	t	t
Cortisol	t	-
Human Chorionic Gonadotrophin	t	+
Prolactin	t	+
Alphafetoprotein	t	t
Ferritin	t	-
Vitamin B₁₂	+	-
Follicular stimulating hormone	-	+
Luteinizing hormone	-	+
Oestradiol-17B	-	+
Progesterone	-	t
Testosterone	-	t

+ Available
- Not Available

TABLE 2
IMMUNOASSAY TESTS AVAILABLE AT THE STATE GENERAL HOSPITALS

	T ₄ (EMIT)*	Opiate screen (EMIT)*	Cortisol (EMIT)*
Kuala Lumpur	t	t	t
Ipoh	t	t	-
Seremban	t	+	-
Penang	t	+	-
Kota Kinabalu	t	-	-
Kuching	t	-	-

* EMIT = Enzyme Multiplication Immunoassay Test

+ available

- not available

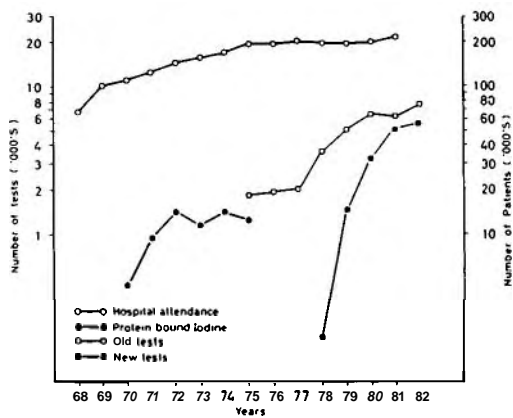


Fig 1: The Radioimmunoassay Workload of Invitro Radioisotope Laboratory, University Hospital.

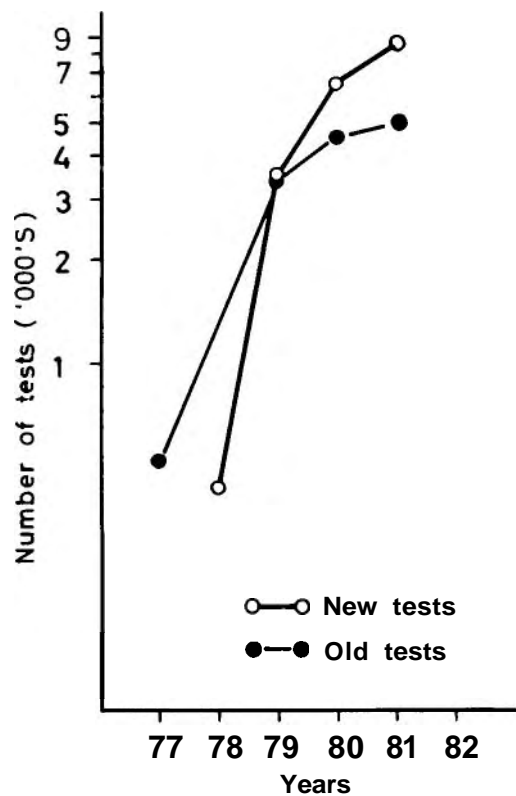


Fig. 2: The Radioimmunoassay Workload of Radiochemistry Laboratory, Institute for Medical Research (Data from Annual Report, Institute for Medical Research, Kuala Lumpur, Malaysia)