

## Chapter 8

# Recent Advances in Immunoassay

R. Edwards, J. Landon and Lesley Rees

*Department of Chemical Pathology, St. Bartholomew's Hospital,  
London EC1, U.K.*

### INTRODUCTION

The astonishing growth of radioimmunoassay (RIA) and related analytical techniques is, in part, due to their sensitivity and specificity — which are inherent in the use of specific, high avidity antisera. Equally important in explaining the successful incursion of such assays into a variety of medical disciplines are their practicality and very wide applicability. Thus a technician trained in a single immunoassay and understanding its theoretical basis can, merely by changing the antisera and labelled antigen employed, determine any of several hundred constituents in a biological fluid.

Even at a time of unparalleled success, it is important constantly to survey the future and assess to what extent immunoassay techniques will continue to dominate laboratory diagnosis during the next decade and ascertain likely changes in methods and equipment used. To this end the presentation is subdivided into a consideration of probable future areas of expansion; the possible impact of non-isotopic immunoassay (NIIA) and developments with regard to work simplification and automation. Other important developments, such as the increasing use of gamma- as opposed to beta-emitting isotopes for labelling, are being dealt with by other speakers at the symposium.

The areas covered in this paper are so wide as to make it impossible to give a complete list of references and, since it would be invidious to select only a few, no references will be given.

### THE FUTURE OF IMMUNOASSAY

The increasing shift in clinical practice from its classical concern with the diagnosis and treatment of established disease towards presymptomatic diagnosis and prevention will ensure continued growth in the use of radioimmunoassay and related techniques. Thus presymptomatic diagnosis, by definition, cannot be based on a clinical history or examination and must depend on the laboratory. Indeed, the number of immunoassays currently performed may represent less than 10% of future demands which will expand as the result both of increased requests for existing assays and the introduction of new immunoassays.

#### Increased Demand for Existing Assays

More than 50 million RIA were performed in 1975, of which about 20 million were for thyroxine ( $T_4$ ). A several-fold increase seems certain even for  $T_4$  assays as, for example, they become mandatory (together with thyroid-stimulating hormone assays) on all neonates, to detect cretinism at a time when therapy can avoid disastrous central nervous effects; the frequency of hyper- and hypothyroidism becomes more widely recognized; and it is appreciated that many patients, especially in the older age group, do not have the

<sup>+</sup>Paper presented by Prof. Landon

classical manifestations but may present, for example, with an unexplained cardiac arrhythmia. Thus routine screening for thyroid dysfunction is becoming increasingly common, especially with the introduction of fully automated RIA and NFA techniques.

Another example is the use of RIA to determine serum levels of alpha foetoprotein (AFP). Thus, while originally introduced to help detect hepatomas, such assays now find their major application in the diagnosis of neural crest lesions during the second trimester of pregnancy. Indeed, their availability offers the exciting possibility that the tragedies of anencephaly or spina bifida may soon become a matter of history. Many other examples could be quoted to indicate likely increased demands for existing assays while it should also be noted that such assays have, until recently, been available to only a relatively small proportion of the world's population.

#### Development of New Immunoassays

During the 1960s, RIA and related analytical techniques were applied predominantly within departments of endocrinology and enabled an explosion of new knowledge in that subject. Immunoassays are having an equivalent effect in many other disciplines, a few of which will be considered briefly.

Clinical pharmacology: Side effects due to drugs are a common cause of patients attending their primary care physician and this realization will ensure a substantial increase in the careful monitoring of drug levels. Several million digoxin determinations are performed annually, in part because suitable assays are readily available. Several other drugs, such as gentamicin, are also toxic and will (once simple, accurate methods are available) also be monitored frequently.

Currently it is seldom known whether failure to respond to a drug is the result of its inefficacy or because the drug has not been taken in adequate amounts or absorbed from the gastrointestinal tract normally. Some drugs, especially those employed in medical oncology, may damage the intestinal mucosa and thereby influence their own absorption. Circulating levels of others, such as the cyclic antidepressants, are known to vary up to twenty-fold in individuals on an identical dose regime. Under such circumstances, careful monitoring to ensure that drug levels lie in the therapeutic range is essential. Immunoassays will also be employed increasingly in pharmacodynamic studies.

Tumour markers: The emotions engendered by cancer guarantee the continued provision of adequate funding for the development of assays for a virtually unending number of tumour markers (Table 1). Owing to their relative lack of specificity it also seems certain

Table 1. Some Tumour Markers Currently Employed in Medical Oncology.

<u>Non-Hormones</u>	<u>Hormones</u>
CEA	HCG
AFP	'Big' ACTH
Gamma foetoprotein 1	AVP
Gamma foetoprotein 2	HGH
Foetal sulfaglycoprotein	PTH
Carcinofoetoglial antigen	$\beta$ -subunit of HCG
Y $\gamma$ antigen	ACTH
S $\gamma$ antigen	Oxytocin
Placental alkaline phosphatase	HPL
SP 1	
Leukaemia-associated antigens	

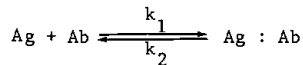
that eventually large numbers of such markers will be determined in each individual sample.

Clinical chemistry: The increasing awareness of the benefits of immunoassay within departments of clinical chemistry may be expected to lead to the gradual replacement of several current assays by immunological procedures. For example, a strong case can be advanced for determining enzymes by radioimmunoassay — since most enzymes are employed merely as a tissue marker and their own biological effects are immaterial. Other suitable candidates for immunoassay are the lipoproteins, bile acids, total and esterified cholesterol, insulin and creatinine (the latter because of the limited sensitivity of present procedures).

Other branches of pathology: In haematology, binding assays for vitamin B<sub>12</sub> and folic acid have already proved their worth, while the RIA of circulating ferritin levels (as an indicator of total body iron stores) is helping to decrease the number of bone marrow biopsies. Immunoassays promise to facilitate greatly the study of clotting disorders and assays for fibrin degradation products, such as fragment E, allow the diagnosis of incipient deep vein thrombosis before any clinical manifestations are apparent. The impact of introducing immunoassays for the screening of hepatitis antigens in serum samples emphasizes the value of these procedures in virology and promises the means of rapid diagnosis and identification throughout all branches of microbiology and parasitology.

Non-medical disciplines: Just as immunological procedures are expanding from endocrinology into other branches of medicine so they will extend from the medical into other scientific fields. There is an obvious need for such techniques in the food industry and in the study of environmental pollution. Already many thousand progesterone assays weekly are being performed on milk as a means for the early detection of pregnancy in cows.

For antibodies and antigen/antibody complexes: While immunoassays are normally thought of as techniques to determine the concentration of an antigen, they can also be employed to detect the presence of antibodies or of antigen/antibody complexes. Thus immunoassay depends upon the specific non-covalent binding of one reactant by another:



where Ag and Ab represent free antigen and antibody respectively and Ag:Ab the bound complex.

Yalow and Berson's pioneering work in RIA followed their use of <sup>131</sup>I-labelled insulin to detect the presence of circulating antibodies in patients being treated with insulin. This approach was later adopted to show that the failure of some children to grow following administration of the early, relatively impure growth hormone preparations was due to the development of antibodies against the hormone. Immunoassays are currently being introduced to enable the rapid detection of antibodies against thyroglobulin and a microsomal fraction from the thyroid, and others will prove invaluable in the diagnosis of various autoimmune diseases. The demonstration of raised levels of specific IgM is being employed for the diagnosis of rubella in the pregnant woman, while the number of assays that will be required for the detection of antibodies against various tropical diseases is incalculable.

Several different techniques are now employed to detect the presence of antigen/antibody complexes and are proving of great value in clinical practice. Such complexes are frequently found following viral or bacterial infections; in patients with autoimmune diseases; and in association with cancer. Indeed, such is the diversity of diseases in which circulating antigen/antibody complexes are found that assays for such complexes may play an important role in health screening. In such screening, the question to be asked is whether the patient is well or ill (as opposed to hospital screening where it can usually be assumed that the patient is ill and the tests are designed to determine the particular organ affected). Current means, such as determining the erythrocyte sedimentation rate or the serum viscosity, are probably less sensitive for this purpose.



addition, the multiplication of the signal upon which EIA depends is also associated with multiplication of any error. Furthermore, non-separation EIA have a limited sensitivity and may lose precision due to the presence, in the sample, of enzymes with similar activity and of factors which may enhance or suppress the activity of the enzyme employed as the label. As a result, the sample volume must be kept small.

#### Respective Roles of NIIA and RIA

It is likely that NIIA, at their present stage of development, will prove complementary to RIA and not have a significant impact on its current market. Thus RIA remains the technique of choice when a precise, quantitative result is required for a hormone or other compound lying in the  $\text{pmol l}^{-1}$  or low  $\text{nmol l}^{-1}$  range. However, the simplicity of non-separation NIIA techniques make them ideal for plus/minus situations (such as the diagnosis of pregnancy or screening for hepatitis antigen positive sera, or for antibodies to various tropical diseases). Such assays will also help open up new markets in countries with severe legal restrictions on the use of isotopes and in the developing world. Their speed and simplicity with regard to the therapeutic monitoring of those drugs (the majority) which circulate in the  $\mu\text{mol l}^{-1}$  or high  $\text{nmol l}^{-1}$  range (thereby requiring only small sample volumes) provides the potential for accurate dose regulation.

Nonetheless, there are fields, such as the assay of serum total  $T_4$  values, in which RIA and NIIA techniques are competitive and this is certain to continue, although not necessarily to the detriment of RIA.

#### WORK SIMPLIFICATION AND AUTOMATION

During the last decade there has been considerable progress with regard to work simplification in RIA: several accurate sample diluters have been developed and systems designed to add the sample (or standard), labelled antigen and antibody; automatic gamma counters are now routinely employed, and several relatively inexpensive systems for handling data are available. Another important recent innovation has been the introduction of a manual multihead gamma counter, by Nuclear Enterprises, which allows the simultaneous counting of 16 tubes containing  $^{125}\text{I}$ -labelled reactant. The value of such an instrument (which has revolutionized the counting habits of the staff in our department) can be illustrated by considering the time taken to count 240 tubes for 30 s. Employing an automated, single head gamma counter this takes about 3 h whereas, with the manual multihead instrument, all tubes can be counted within 10 min.

The introduction of fully automated systems will, by improving precision, increasing throughput and reducing costs, have a significant impact — particularly for those assays for which demand is already considerable. Some instruments offer the additional advantage of not requiring equilibrium to be reached (in contrast to manual procedures) thereby shortening assay times. Manual procedures will, however, always be required both as a back-up to automated systems and for those determinations for which the number of samples does not justify automation. Clinical chemists, who have witnessed the introduction of automation into the routine laboratory, will experience a feeling of déjà-vu. One can expect long discussions concerning the relative merits of discrete and of continuous flow analysers; there will be a progressive increase in sample throughput and, finally, multi-channel systems will be produced. Obvious applications for the latter include feto-placental assessment, screening for tumour markers and the laboratory diagnosis of thyroid disorders.

Recent experience with multichannel screening in the routine clinical chemistry department has been disappointing from the standpoint of patient diagnosis. There is, however, a fundamental difference between screening as currently performed and the type of screening which will be made possible by multichannel immunoassay systems. Thus, at present, the assays provided are those whose chemistries can be included within available automated systems. In the future, the wide applicability and flexibility of immunoassay will help ensure that the laboratory tests performed are those which the clinician requires and which fulfil the essential criteria of such tests — namely that the disorders being screened for are relatively common; that there are few false positives and negatives and, of particular importance, that suitable treatment is available.

## CONCLUSION

It is concluded that the number of immunoassays currently performed will continue to grow rapidly because of increasing demand for existing assays and the continual introduction of immunoassays for new compounds. Others have predicted a levelling off at some 70 million after 1983, due to alternative NIIA becoming available. Such forecasts are, of necessity, based upon the situation existing at the time of the forecast, in terms of both clinical practice and available technology. The recent wide acceptance of testing neonatal blood spots to diagnose cretinism and assays for alpha fetoprotein to detect neural tube defects indicate the problems faced in such calculations. Thus, many of these assays lend themselves only to RIA due to their low circulating levels or the small samples that can be obtained.

It is our opinion that the overall impact of NIIA methods will, in the short term (five years), be less than many expect especially since many laboratories have made (and, with the advent of automated systems, will continue to make) heavy capital investment in RIA. NIIA will, however, be used in new situations such as drug monitoring and plus/minus diagnoses, thereby opening up their own areas of application.

## DISCUSSION

S.J. BREWER: The availability of receptor proteins isolated from the target organ of some hormones and drugs avoids one major difficulty with RIA, namely the raising of specific antibodies. Could you comment on the advantages of such binding proteins and on their use?

J. LANDON: There has been considerable research, worldwide, on the use of specific receptor binding proteins for assay purposes. However, present evidence suggests that such binding proteins will not replace the use of antibodies. Their isolation is frequently complex and once isolated and purified they frequently prove unstable. They do not appear to offer significant advantages with regard to specificity and sensitivity as compared with the use of antibodies and, of particular importance, the number of compounds for which appropriate receptor proteins can be isolated is relatively small. This contrasts with the very wide applicability of immunoassays since antibodies can be raised against an immense range of haptens, peptides and proteins.

V. MARKS: The future of clinical biochemistry is probably a return to the bedside (or clinic) so that the result of an analysis can be obtained virtually immediately, allowing effective action to be taken. What methodology and instrumentation does Professor Landon envisage to be the technology of this future development?

J. LANDON: It would seem likely that immunoassays will eventually play an important role both at the bedside and in the outpatient clinic. Thus the time taken for an immunoassay to reach equilibrium is related to the concentration of the reactant and with, for example, many drugs which lie in the  $\mu\text{mol l}^{-1}$  range results can be obtained within minutes. There would be obvious advantages in employing one of the many alternatives to an isotope for labelling and relatively inexpensive and simple instrumentation is now being developed for their end-point detection within an outpatient department.

P. ROSS: Enzyme immunoassay requires the coupling of a hapten to an enzyme without loss of enzyme activity. Is this a difficult procedure in your experience?

J. LANDON: Several techniques are available which allow such coupling to be made without loss of enzyme activity. There are also assays such as that introduced for thyroxine ( $T_4$ ), by Syva, in which use is made of the fact that there is a loss of enzyme activity when malate dehydrogenase is covalently linked to  $T_4$ , which is restored when the  $T_4$  is bound by a specific antibody. Thus loss of activity may be made useful, provided the binding releases this loss of activity.

D.M. TAYLOR: Do you believe that large scale screening type assays should be performed in district laboratories or at specialist supraregional centres?

J. LANDON: The United Kingdom has established a national service designed to ensure that radioimmunoassays and related techniques used for clinical purposes are provided

at the most appropriate level. Some are performed at large supraregional centres — including complex, seldom requested assays (such as those for ACTH) and, possibly, in the future, very high volume screening assays including, for example, those for alpha-foetoprotein in early pregnancy and to determine the neonatal thyroid stimulating hormone levels. Other assays such as those requiring urgent results (like digoxin) must be performed locally while others, such as LH and FSH, may be best performed at a regional or area level.