# The promotion of radioimmunoassay in human health

by R.A. Dudley*

The Agency's promotional role in the application of radioimmunoassay to human health is simply summarized: to help developing countries establish effective radioimmunoassay services, and to encourage the application of radioimmunoassay techniques to those health problems in which they can make the greatest contribution. The Agency's expertise is related more to techniques than to medical problems, and when medical issues are paramount the Agency collaborates closely with the medical community, in particular with the World Health Organization. And in the spirit of technical co-operation, the Agency is guided by the wishes of its Member States. But however circumscribed, the Agency's role can be challenging and exciting: the nature of radioimmunoassay ensures this.

Radioimmunoassay is an analytical technique pioneered by medical physicists 25 years ago, and it has so revolutionized the biochemical analysis of living systems that one of the pioneers, Dr Rosalyn Yalow, was awarded the 1977 Nobel Prize in Medicine for her share in it. A recent report in *IAEA Bulletin* Vol.24, No.4 (December 1982) on an Agency symposium summarized some of the attributes of this technique.

Life is made possible by the balanced interaction of thousands of complex organic molecules, whose sizes range from a few to many thousands of atoms, and whose concentrations in biological fluids range from parts per hundred to parts per billion. Many differ from each other almost negligibly, yet nevertheless have very different functions. Before the advent of radioimmunoassay the assay of most of these substances was difficult or impossible, and insight into biochemical processes was correspondingly restricted. Radioimmunoassay brought two key innovations. First, it introduced as a highly specific and sensitive "reagent" a particular class of biological molecule, namely antibodies, to segregate the particular substances of interest. Second, it made use of radioactive tracers to permit quantification of minute amounts of these segregated molecules. The latter characteristic is responsible for the Agency's involvement with the technique. Without going into details, it can easily be seen that chemical analysis under such extreme conditions is fraught with pitfalls whose elimination has been the goal of intense methodological development.

To set this article in context, radioimmunoassay is just one of several medical applications of radionuclides; however, it is the one that can be carried out at the lowest level of technical complexity. Secondly, radioimmunoassay is equally applicable to studies in animals; the Agency's substantial programme in that field will not be touched upon here. And thirdly, a whole range of related procedures has been spawned by radioimmunoassay, some of which use specific biological "reagents" other than antibodies, and tracers other than radionuclides. Indeed, such tracers offer advantages in many circumstances, with the result that radioactivity may play a diminishing role in this field. Nevertheless, radioactive tracers will not disappear, and since much of the expertise developed with one class of tracer is immediately relevant to others, the lasting value of the Agency's promotional efforts is assured.

Radioimmunoassay and related techniques have come to play an enormous role in medical diagnosis and research. Today hundreds of different substances are assayed by these methods, including hormones, vitamins, pharmaceuticals, drugs, products shed by infectious viruses and by parasites, substances released by malignant tumors, and many others. The yearly investment in these techniques, world-wide, must exceed US $1 billion, and the techniques play some part in the diagnosis of perhaps 10% to 20% of all the patients hospitalized in developed countries. It is natural that developing countries are eager to introduce these methods into their own health systems.

### Four categories of radioimmunoassay laboratory

The public health problems of most developing countries are very different from those of developed countries, and it cannot be assumed that the role and organization of radioimmunoassay services in the first may be directly copied from those in the second. Various advisory bodies have identified for the Agency and the World Health Organization (WHO) some of the issues that must be considered.

Proper organization of radioimmunoassay services is essential to their success. In the first place, they cannot thrive in isolation, but must grow in consonance with associated laboratory services, especially those of the clinical chemistry laboratory. Secondly, it is desirable that these services develop in a rational hierarchy of laboratories. An IAEA advisory group conceived a hierarchy of four categories: the lowest category

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laboratory would perform analyses of the most common substances using acquired reagents, but would still be responsible for quality control and interpretation of its own results; laboratories in the next two categories would label some reagents for their own use or for wider distribution; and the top category laboratories would engage in more extensive preparation of reagents, quality control, training, and general support to the system. This division of labour should increase efficiency and reduce cost. However, since samples for analysis can be easily transported, most work can be concentrated in only a few laboratories.

The quality of the staff in each laboratory will be a key to its effectiveness. A laboratory in the lowest category might have only a single technician, who would however receive analytical support from the associated laboratories and support for interpretation of the results from a medical graduate. Staffing at higher levels would depend on the workload, but would require higher levels of medical and chemical expertise.

Contrary to widespread opinion, the facilities required in radioimmunoassay laboratories can be comparatively simple. At the lowest level, one or two small rooms would provide adequate space. A manual well-scintillation counter costing US $1000 could cope with 100 or more samples per week. Ancillary equipment, including a centrifuge, radiation monitor, small programmable calculator, freezers, pipettes, and various minor items would bring the total cost to a figure below US $10 000. In higher categories of laboratories, a fume hood would be necessary for labelling reagents with radionuclides, an automatic counter costing US $10000 would be highly desirable if the workload ran to hundreds of samples per week, and additional items such as fraction collectors and chromatography apparatus would be needed according to scope and quantity of work. Assured supply of reagents is essential. Most of these are common laboratory reagents, costing perhaps US $0.10 per sample. The distinctive radioimmunoassay reagents, however, are in a special class. Most small laboratories purchase these as commercial kits, for which costs can be as high as US $10 for duplicate analysis of one sample. Local preparation of reagents is feasible, but requires greater expertise. When many samples are to be analysed, such local production can reduce costs to US $1 per duplicate sample and lower.

A rigorous quality control programme is indispensable. This is neither expensive nor technically complex, but requires a continuing commitment and discipline. It involves, as part of the laboratory's internal programme, the standardization of conditions, frequent assay of appropriate reference substances, and critical analysis of the resultant data. It should also include routine analysis of reference preparations, having concentrations unknown to the laboratory, which are distributed by some external body to monitor quality in the country or region.

Finally, it is obviously essential that the potential applications of the technique be carefully chosen in order to obtain the maximum yield in improved health for each dollar spent. The natural tendency is for laboratories in developing countries to measure the same substances as laboratories in developed countries, since the necessary methods and reagents have been made available. Foremost among these substances are hormones, especially thyroid and reproductive hormones. Analysis of blood for infection with hepatitis B is almost universal in blood transfusion services in developed countries, and illustrates the potential role of these techniques in the management of transmissible diseases. Many more such diseases are prevalent in developing countries, e.g. malaria and other parasitic diseases, or tuberculosis and other infectious diseases. However, since such problems have not required major attention in the medically advanced countries, it is not yet established that radioimmunoassay techniques can play a decisive role in their investigation or management. This stands as an exciting challenge for the immediate future. And finally, it is likely that the most rewarding applications of radioimmunoassay are not to be found in diagnosis of patients' illnesses, but rather in research into the nature and cause of diseases, thus leading to their control.

**Nearly US $2 million spent in three years**

The foregoing considerations suggest how the mechanisms of Agency support should be focused. The scope of these activities can be illustrated by the programme during the period 1980 to 1982, for which approximate figures are summarized below.

By far the largest support has been channelled through the various activities of the IAEA's Technical Co-operation programme. During the three-year period, equipment and experts were allocated to 16 countries, through a total of 21 projects, including those financed from funds either of the Agency or of national donors. A total of about US $570 000 was spent on equipment (about US $35 000 per project). Approved expert missions totalled in value about US $230 000, corresponding to roughly 50 man-months. The major share of these expert services has gone towards the improvement of general radioimmunoassay competence within the host laboratories, although occasionally such services have been devoted to the introduction of special techniques. The bulk of this effort has been related to methodology in clinical diagnosis.

During the same period, about 45 fellowships were approved, mainly for physicians or clinical scientists, but also for a small number of technicians. The total duration was about 300 man-months (average of 6 months per Fellow) and the total cost about US $500 000. These Fellows came from about 25 countries; about 280 of the 300 man-months were completed in Europe or North America, while most of the rest were in Latin America.

The Agency organized two training courses in radioimmunoassay during the period 1980 to 1982. The first, in the German Democratic Republic and Poland, was attended by 18 participants for a period of 5 weeks while the second, in Bulgaria, had 13 participants for 3 weeks. The funds allocated to the two courses together totalled US $150 000, of which the major part was spent on travel from all over the world. A third course, concerned with the use of nuclear techniques in the study of parasitic infections, also included a section on radioimmunoassay.

Taken altogether, the technical co-operation programme represented a commitment to radioimmunoassay of almost US $1.5 million during the period 1980 to 1982. Expert services, fellowships, and training courses provided about US $900 000 toward development of human resources, as compared to the US $570 000 committed for equipment.

Next to technical co-operation projects, the research contract programme has represented the largest allocation of funds: US $270 000 to 68 projects in 32 countries during the period in question. Probably the larger part of these funds was devoted to purchase of equipment, although supplies and staff salaries contributed significantly to the total. Typically such contracts extend over a period of three years, and are organized in groups of co-ordinated programmes with a common theme to each. A few examples are discussed later. In most cases the research has been directed toward the introduction of techniques new to the laboratory, and their application to diagnosis or medical investigations of local concern.

Information exchange is another activity of major importance, although much of it, through informal staff contacts, cannot be subjected to accounting. The most visible activities are symposia. The 1982 Symposium on radioimmunoassay and related procedures in Medicine was held in Vienna at a cost to the Agency of US $30 000, and was attended by 239 participants and observers from 49 countries. The published proceedings, containing 76 presentations, display both the state of development of this field in individual laboratories, and a broad picture of its current scope and direction. Another symposium, of comparable size and held in Vienna in 1981, on nuclear techniques in the study of parasitic infections, also dealt extensively with radioimmunoassay procedures. Additional funds totalling perhaps US $100 000 have been devoted to smaller groups of experts, consultants, or meetings of the participants in the co-ordinated research programmes.

**Maintenance and training important**

As the foregoing programme has evolved, it has stimulated many questions as to how its effectiveness could be improved. These have led to a number of projects that may favourably shape its future.

One possible anomaly that has become apparent is the comparatively large cost of equipment, e.g. an average of US $35 000 for each individual technical co-operation project, when projections have suggested that good work at a modest volume can be accomplished with equipment costing in total US $10 000 or, even with an automatic sample counter, US $ 20 000. Could output of equal quantity and quality be achieved with lower expenditure on equipment? One prominent issue is the life-expectancy of equipment, i.e., the effectiveness with which it is maintained so as to ensure that its early replacement will be unnecessary. The Agency has started a programme utilizing research contract and technical co-operation resources to determine how well equipment is now being maintained and to stimulate more effective maintenance through better conditioning of the climatic and electrical environment, better training of technicians, and a more careful approach to preventive maintenance. (See the article by Mr Vuister on page 24 of this *IAEA Bulletin.*) In addition, the Agency's laboratory has designed an automatic gamma counter which is now being manufactured commercially. The idea is to make available an inexpensive instrument that is more easily maintained locally and is less vulnerable to disturbances in the electrical power system. (See the article by Mr Dudley on nuclear medicine and the electronics revolution on page 30 of the *IAEA Bulletin* Supplement 1982). Steps are being taken to encourage the assembly or manufacture of similar counters in countries having the need and resources.

Another matter is optimum training in radioimmunoassay. Training courses are very expensive compared with fellowships (US $5200 vs. US $1700 per man-month of training), for the elementary reason that the high costs of travel are amortized over a shorter period of training. Moreover, this short period of training means that participants must be able to understand fully the language of the course right from the outset, a condition that is often not met. In collaboration with WHO, the Agency is therefore improving the efficiency of these training courses. The objective is to devolve the courses to a more local level, thus insuring a big reduction in travel and perhaps even subsistence costs, and allowing instruction in a language familiar to the trainee. To make this approach feasible, a model syllabus, various textual and audio-visual training aids, and perhaps kits of reagents and equipment for pratical exercises, will be developed; and their use demonstrated in courses to which prospective trainers from various regions will be invited. When these trainers in turn organize local courses using these same materials, the need for foreign input as well as for travel of trainees should be greatly reduced.

Experience has shown that developing countries often lack a tradition of quality control and that the added expense of such activities or the unavailability of the

necessary reference materials hinders the growth of such a tradition. The Agency has taken several initiatives to stimulate greater attention to quality control. One project has been a co-ordinated research programme on internal quality control in which over 40 laboratories have participated. The emphasis has been upon the introduction of more perceptive data processing, using an inexpensive calculator and purposed-designed calculator programs developed by Agency staff. In addition, pools of serum were supplied, allowing analysis of a common material in successive assay batches and thus a check on reproducibility. Collectively these practices should make more apparent the degree of reliability of the assays, and the nature of limitations thereon, so far as scrutiny within the laboratory is capable of revealing this information. A second project has been the organization of external quality control programmes. About 90 laboratories participated in the first such scheme, wherein a central laboratory distributed for assay on a monthly basis a series of serum samples reflecting various states of thyroid disease, and thereafter collated and distributed the reported results. A follow-up project is now being formulated so as to permit a continuing external quality control of thyroid-related hormones via a number of national or regional centres. The objective of all these quality control activities is to reveal the present errors in assay practices, to suggest how they can be eliminated and generally to bring the reliability of the hormone analysis into harmony with the clinical requirements.

Several evaluative studies have been initiated under research contracts critically to assess the potential role of radioimmunoassay in various applications. One such study was a systems analysis of alternative methods of screening transfusion blood for hepatitis B, taking the conditions of India as a model for developing countries in general. Another project, just starting under a co-ordinated research programme, is the investigation of strategies appropriate to the study of thyroid disease in developing countries. Many diagnostic procedures are available, not least the clinical examination. However, radioimmunoassay of various thyroid-related hormones is widely used and can yield nearly decisive conclusions. The goal is to determine in what combination and sequence these various tests can best be performed in the circumstances prevailing in developing countries.

**Special applications for developing countries**

And finally, the hope is widespread that more important applications await the attention of radioimmunoassayists in developing countries than merely those diseases that are conventionally studied in developed countries. A particular such class of application is the investigation of parasitic diseases, for example filariasis, malaria, and schistosomiasis, and many laboratories interested in tropical diseases have recently taken up this problem. In a co-ordinated research programme, the Agency is assisting one group of laboratories in both developed and developing countries to test the techniques under conditions allowing maximum input of available expertise under circumstances found where the problem is greatest. Many believe that breakthroughs in the control of these diseases are imminent, and that the techniques of radioimmunoassay and related procedures will play a role in these developments.

Radioimmunoassay and related procedures will almost certainly have major roles to play in the improvement of health in developing countries, as they do in developed countries. Experience will clarify what these roles are, but they will undoubtedly encompass both diagnosis and research. The Agency's assistance, small though it is on an absolute scale, contributes valuably toward the establishment of the necessary technical expertise.