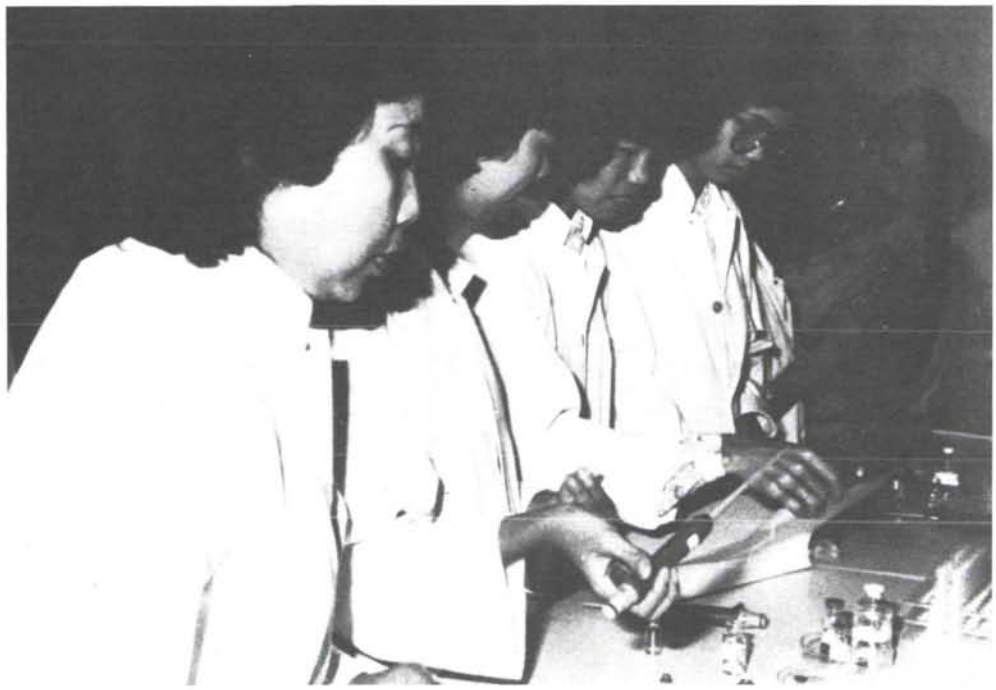
At an IAEA regional training course for Asia and the Pacific, participants are instructed on the use of computers and data processing programs for reliable analysis of RIA.

Participants from 16 countries were trained in RIA methodology at an IAEA regional course held in Indonesia.



Radioimmunoassay for human health in developing countries

Through IAEA co-operative projects, countries have been able to solve some basic problems

by R.D. Piyasena, P.L. Airey, R.D. Ganatra, and M. Nofal

Radioimmunoassay (RIA) is a microanalytical technique where radionuclides are used in diagnostic tests for the measurement of minute quantities of substances, such as hormones, vitamins, and drugs, in body fluids. The distinct advantages of sensitivity and specificity that the technique offers enable such measurements in small sample volumes, and in most cases without cumbersome extraction and purification steps. Measurement of radioactivity, as the end point, is more accurate than chemical estimations.

Since first introduced in the early 1960s, RIA has gained wide acceptance as an analytical method adopted by an increasing number of developing countries as an appropriate technology that can be managed within the capabilities of local infra- structures. An informed estimate would be that there are, at present, in excess of 500 hospitals, university, or other laboratories in the developing world engaged in RIA on some scale.

In the developing world, RIA is used primarily for patient management, but research activity is also increasing as expertise and resources improve. The majority of patient samples processed are in relation to thyroid disorders, with RIAs for thyroxine (T_4), triiodothyronine (T_3) and thyrotrophin (TSH) outnumbering other procedures. However, the technique also is used widely in the investigation of other endocrine conditions and public health problems. To an encouraging degree, some developing countries have gained the capability to perform radioisotopic microassays in areas of clinical and research importance such as steroid receptor quantification in breast tissue; diagnosis of bacterial and parasitic disorders; investigation of infertility and sterility; narcotic drug abuse; and organ transplantation.

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Building the infrastructure

Through its nuclear medicine (formerly medical applications) section of the Division of Life Sciences and the Division of Technical Assistance and Co-operation, the IAEA has served as a catalyst for RIA development in many countries, often pioneering its introduction. Efforts are mainly directed at promoting the transfer of RIA technology to developing countries by helping laboratories to obtain low-cost reagent supplies and to monitor the quality of the assays.

The emphasis has been on the creation of the necessary infrastructure, based on an appreciation of existing problems and constraints, so as to enable RIA to fulfill its potential in serving health care needs. Since RIA is a multidisciplinary function, its introduction has to be considered in conjunction with the availability and state of development of other laboratory disciplines. Template requirements are equipment, training, and reagent supplies. Additional activities involve the maintenance of assay quality and consistency.

Equipment. A reliable gamma scintillation counter is essential for an RIA laboratory. The simple single-well, manual scintillation counters initially used, even in industrialized countries, have given way to automatic gamma and liquid scintillation spectrometers.

Costs, however, have risen in line with the equipment's sophistication, and by the early 1970s, the provision of counters for RIA was not possible on a scale commensurate with demand. To improve this situation, the IAEA contributed to the wider use of a relatively inexpensive gamma counter with data processing capability that was manufactured commercially. The IAEA has assisted in supplying this system to several RIA laboratories in the developing world.

Relative to increases in equipment costs for *in vivo* nuclear medicine, such as gamma cameras, RIA counting equipment has actually decreased in cost in recent years. Multichannel gamma counters, when required,

are now selected from several commercial models. Liquid scintillation spectrometers are only exceptionally supplied, since an increasing number of substances, including steroids, are now assayable using iodine-125.

Other equipment needs for basic RIA are simple. The major item is a suitable centrifuge. Experience with RIA centrifuges has been mixed, and some have broken down in situations where local repair is not possible. Cognizant of this, the IAEA has taken advantage of modern developments that render RIA methods less dependent on a centrifugation step.

Training. The IAEA attaches high priority to an adequate personnel resource base for RIA projects. Trained personnel are required in two broad areas: in the techniques and methods themselves, and in the servicing, maintenance, and repair of RIA instruments. In support of each area, the IAEA provides expert services and group and individual training, and organizes seminars and symposia.

As a general rule, whenever an RIA facility is set up anew, or upgraded, in a developing country, the services of an expert are provided for a suitable period. The experts ensure that equipment is correctly installed and introduce relevant techniques. The training of counterpart staff is always considered important. Approximately 100 expert missions have been carried out since 1980, the majority in the Asia and Pacific region and in Latin America (35–40 in each region), about a dozen in Africa, and the rest elsewhere. In many countries, RIA has yet to be introduced. Where it has been, however, expert missions have made achievements in the upgrading of RIA techniques and stimulating local reagent production.

Group training activities such as interregional and regional training courses and workshops have been effective in creating and upgrading expertise. Such courses are now organized at a "train-the-trainers" level, with international teaching staff. Recipients further disseminate the knowledge gained at follow-up national or local courses overseen by the Agency. The approach has proved fruitful and cost effective. For example, two regional courses in the Asia and Pacific region in 1987, each drawing attendance from 16 countries, dealt with the topics of bulk reagent methods for RIA, quality control, and data processing. Follow-up national courses have resulted in more than 10 times the number of participants receiving the same training locally.

Individual training fellowships are available under the IAEA's technical co-operation programme. At the academic level, they are usually made tenable in advanced laboratories. An average duration would be 3 months to a year, and the choice of host institution is made to conform to the trainee's needs. The programme has resulted in the majority of Member States, particularly in the Asian and Latin American regions, having key RIA laboratory personnel trained by IAEA. In addition, senior scientists have benefited from Agency-sponsored scientific visits, of up to 8 weeks duration, for familiarization with techniques in areas of special interest.

Specialists and workers in developing countries have further been kept abreast of advances in the field through seminars on specific topics, and symposia on radioimmunoassay and related procedures in medicine that have been organized about every 5 years since 1970. The meetings also provide a forum for presentation of their own work and the opportunity for discussion with colleagues.

Another programme, built over the past decade, is designed to support maintenance of instruments, and directed at promoting self-sufficiency in this area. Assistance extends to testing equipment, providing experts, and conducting training activities.

Solving supply and cost problems

Costs, especially when they have to be met from scarce foreign exchange, have been an obstacle to the progress of RIA as well as other technologies in developing countries. The essential problem is that reagents have had to be purchased from abroad, most often in the form of commercial kits. Most of the kits are of good quality and convenient to use, resulting in wide usage. In 1987, out of more than 120 laboratories participating in an IAEA project in the Asia and Pacific region, only three were not totally dependent on them.

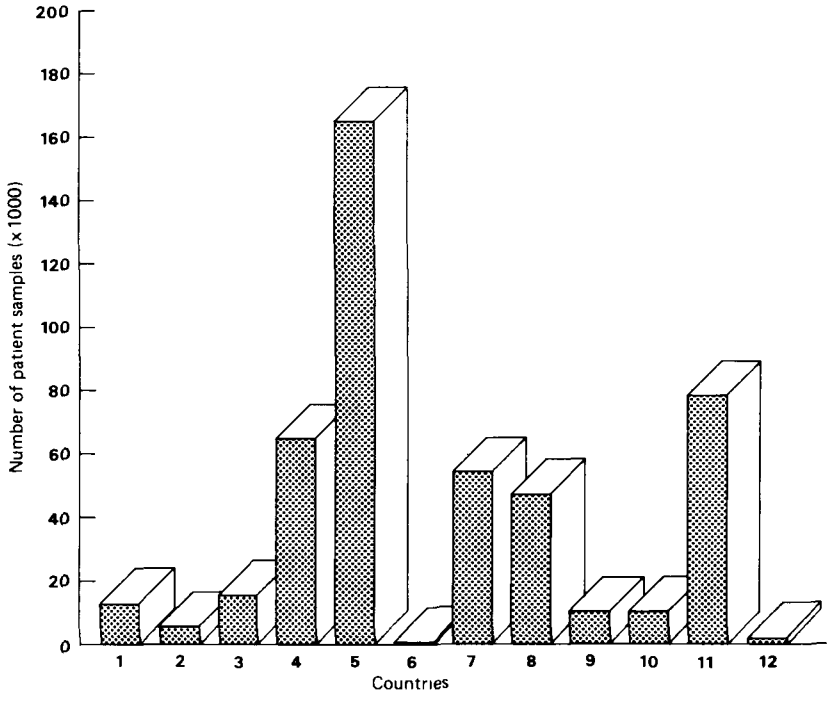
Several disadvantages, however, have begun to exert a negative influence, particularly when clinical demand has increased. Most laboratories in the developing world find themselves without the financial means to purchase RIA kits in required quantities, resulting in the RIA service becoming highly selective. For example, assays fairly commonly needed, such as for TSH, are either unavailable or done at unacceptably low frequency. Of the laboratories in the Asia and Pacific region, all were providing an RIA in 1987 for T_4, most for T_3, but less than half had a TSH assay at all.

Apart from high costs, other deleterious factors to the development of RIA include uncertain logistics of supply, customs delays, and poor storage conditions. The net result has been a reduction in the quantity of RIA service relative to demand and, more seriously, in quality as well.

Many laboratories, attempting to make do with what was on hand, have resorted to practices — for example, neglecting duplicate sampling and disregarding standard quality control procedures and manufacturer's protocols — that severely compromised assay quality. Only 38 of the 120 laboratories in the Asian region were implementing quality control practices in 1987 that could be considered as adequate. All others had no or rudimentary quality control procedures.

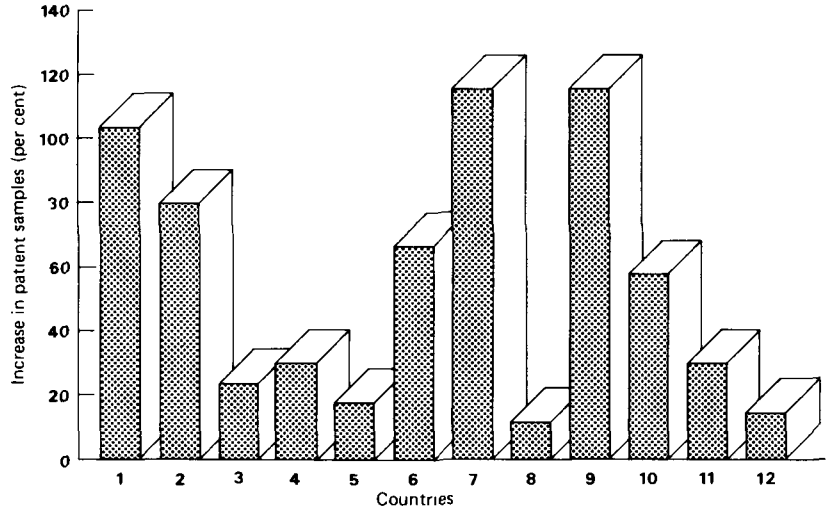
Steps have been taken towards solving this problem. Two IAEA projects in Asia and the Pacific and in Latin America initiated in the mid-1980s are designed to deal

Number of patient samples assayed for thyroid hormones in 1987 in Asia and the Pacific



Under an IAEA regional project, laboratories were able to dramatically increase their assays of patient samples following the introduction of a low-cost supply scheme.

Growth in assays of patient samples, 1986–87, in Asia and the Pacific



primarily with the issue of improving sources of reagent supply. Taking advantage of the availability of RIA reagents in bulk at reduced costs, the initial project strategy was to procure them from a central source and encourage the introduction of "in-house" assays based on their use. Assays for thyroid-related hormones were selected as being the most common.

Under the projects, nearly a million tubes of a complete package of bulk reagents for RIA of T_3 and T_4, and an immunoradiometric assay for TSH, have been supplied from a central source to more then 150 laboratories in the Asia and Pacific and Latin American regions. An intensive training programme through regional courses, followed by others at the national level, has been initiated to introduce the new methodology and procedures for internal quality control and modern data processing. Results have been closely monitored by the IAEA.

Supply of reagents at project cost — through a system of national co-ordinators — was done for 12–18 months. Even within this time, the projects exerted a profound impact on three vital areas — the introduction of new technology, decrease in costs, and increase in workloads. By the end of 1987, some progress was already evident: Every participating country in Asia and the Pacific had acquired the TSH assay at least in its main

centres. The same became true in Latin America early in 1988.

The effect on costs has been dramatic. From reliable estimates, the reagent cost of assaying a single patient sample was reduced by a factor of about five, to less than US $0.50, from a previous average of US $2.50. An added advantage has been adherance to standard RIA and internal quality control practices previously neglected.

As the procedures became less expensive, their use expanded to satisfy clinical demand and workloads were at times seen to double in a single year. For example, the total number of patient samples processed for thyroid-related hormones in six participant laboratories in an Asian country in 1986 was about 25 000. The figure provided for 1987 is 55 000. In another case, the number of patient samples assayed for TSH increased from 120 in 1986 to 1000 in 1987. In a third instance, workloads increased over those years from 6800 to 18 000 patient samples.

Promoting self-sufficiency

These regional projects were intended to promote self-sufficiency within each participant country to an extent scientifically and economically meaningful. Accordingly, local reagent production was systematically stimulated following the recommendations of an expert panel.

Regional training courses have contributed greatly to the success of this venture. Of the 120 laboratories in 16 participating countries in the Asia and Pacific region, only 10 (five in one country) were using a locally produced antiserum for T_4 RIA in 1987. Eight laboratories (four in one country) prepared their own tracers, 11 their own standards, and 18 their own quality control sera.

In the case of other laboratories participating in the projects, dependence on imported materials was drastically reduced by early 1988. By then the T_3 and T_4 RIAs were being done using reagents that were "home grown" (except for iodine-125 for tracer preparation) or obtained from a production centre close at hand. The situation in regard to reagents for the TSH immunoradiometric assay is not yet satisfactory, but active steps towards improvement are presently in train.

Participating laboratories in several countries are now reaching self-sufficiency in reagents for T_3 and T_4 RIA, while others progress in this direction. In some cases, locally produced reagents are being used, after proper testing at internationally recognized centres, only on a national scale. Reagents from other centres, however, have been distributed regionally. They have been found quite acceptable, and it is expected that the activity would be enhanced.

Besides the scientific achievement, the main practical advantage is decreased costs; the materials themselves are cheaper and savings are realized in transport charges. For example, in an Asian country, a 100-tube assay for T_4 or T_3, using commercial reagents, costs the equivalent of US $180. The same quantity, with reagents produced locally, is offered at US $45. With the increased participation of other countries in 1989, it is expected that regional "indigenization" of reagent supplies and formalization of regional distribution schemes would cause reagent costs to even fall under the US $0.50 per sample achieved in 1987.

Signs of similar success are apparent in the Latin American project, which began a year after the Asian one. It is now at the stage of introduction of bulk reagent-based methods and quality control. However, local reagent production is already at a fairly advanced stage in some countries.

Quality and reliability assurance

Standard quality control procedures in RIA have been, generally speaking, conspicuous by their absence in the majority of laboratories in developing countries for two main reasons. First was a lack of knowledge, an appreciation of how necessary they are in RIA which, for all its advantages, has an inherent fragility deriving from the limited stability of the reagents. Second was the factor of cost. Every sample devoted to quality control meant one less patient sample.

In IAEA projects, a totally uncompromising policy was adopted to deal with this problem. With the introduction of bulk reagent-based methodology, the cost factor as a constraint to quality control was removed, but the essential issue of education remained. The subject was emphasized at meetings of national co-ordinators and regional training courses, where minimum standard RIA practices were agreed upon. Adherance to these, with regular reporting of results, rather than to the particular type of RIA methodology adopted, was taken as the criterion for participation and continued IAEA support. The ideas gained wide acceptance. Although the situation is not as yet completely satisfactory, there has been much improvement. No laboratory has dropped out or been excluded from projects on the grounds of inattention to quality control.

Computer-assisted methods for RIA analysis. Motivation towards and adoption of quality control practices were facilitated by a parallel development that introduced computer-assisted methods for the analysis of RIA and internal quality control data. Data processing packages have been commercially available at least since the early 1970s. But they were expensive, and computers at that time were generally beyond the means of developing countries.

Among the first significant contributions having a visible impact was the IAEA's development of a set of data processing programs. They incorporate advanced concepts but operate on an inexpensive programmable

calculator that interfaces with a gamma counter. By the mid-1970s, this system was being made available at a price well below that of commercial models.

Such a data processing system that enables proper statistical evaluation of results is a great asset to the monitoring of assay performance. When properly used, it also serves to promote good RIA and quality control practices. However, though satisfactory from a technical viewpoint, the calculator's limitations meant slow operation of data programs. In 1987–88, the IAEA upgraded the programs to take advantage of the greater computing power of personal computers, which are now more affordable. The programs already have been used at several training courses and distributed to about 100 RIA laboratories. About 50 laboratories also have been supplied with computers recently under various IAEA projects. Currently, computers and data processing systems are available in almost all countries in Asia and the Pacific and in Latin America, at least at major centres, and are being enthusiastically used.

RIA for research in developing countries

In common with other medical disciplines, RIA in developing countries has overwhelmingly served patient care. Where lack of equipment, trained manpower, and reagent supplies have been a constraint to fulfilment of this function, the impediment to research has been even greater. Direct support for research by IAEA is most commonly through co-ordinated research programmes confined to a few participants, and through research grants to individual institutions.

The growing use of RIA techniques at reduced cost has increased research possibilities through various mechanisms. Research into thyroid-related disorders has been stimulated in many countries, particularly where skills were available but means were few. As cases in point, several articles have been published recently in prestigious journals by authors from laboratories in the Asian region where bulk reagent methodology obtained under IAEA projects is being employed. Independent and locally supported research into iodine deficiency disorders has been initiated in some countries. About 10 regional laboratories in Asia have been carrying out a clinical trial of a strategy for *in vitro* thyroid function testing since 1988.

Future plans and developments

Experience over the past 3 years has shown that an integrated approach to projects on a regional basis is successful and cost-effective where countries share common problems. Moreover, the projects were able to bring together a team of well-trained and motivated workers. The spirit that now exists can be fostered and exploited in several areas, most notably in sharing reagent supplies.

In the Asia and Pacific region, an external quality assessment scheme for thyroid-related hormones will be established in 1989 at a regional level, with perhaps an interregional component as well. A training course will deal with the optimization of local reagent production activities, and formalize the regional reagent distribution scheme initiated in 1988. The RIA data processing programs are being completed in an "on-line" version. A major innovation will be the introduction of RIAs that do not include a centrifugation step (such as magnetizable particle-linked systems) for laboratories lacking such an instrument. The local availability of the required materials, a recent development in itself, makes this an immediate realistic activity.

Thyroid-related hormones have been chosen for the regional projects as a first example. There has been a strong demand from national co-ordinators that similar regional projects be organized in areas of other major health problems. The present infrastructure, including trained workers in adequately equipped laboratories already co-operating with each other, can thus be used to the best effect. Specific proposals are under consideration.

Overall, RIA is expected to have a long life span in developing countries requiring an appropriate and practical technology for health care and research. Techniques are becoming simpler and of more universal applicability, so horizons of RIA could broaden. The IAEA's promotion of RIA, with its sound track record in developing countries, will be needed through the challenging times ahead.