Invited Paper

PRODUCTION OF PRIMARY REAGENTS FOR RADIOIMMUNOASSAY IN DEVELOPING COUNTRIES
Problems and solutions

P. BARTOLINI
Division of Medicine,
Department of Application of Nuclear Techniques in Biological Sciences,
National Nuclear Energy Commission (IPEN-CNEN),
São Paulo, Brazil

Abstract

PRODUCTION OF PRIMARY REAGENTS FOR RADIOIMMUNOASSAY IN DEVELOPING COUNTRIES: PROBLEMS AND SOLUTIONS.

The production of radioimmunoassay (RIA) and immunoradiometric assay (IRMA) reagents in developing countries is analysed in the light of the experience acquired in IAEA organized programmes such as ARCAL VIII for the production of thyroid related hormone assay reagents, and the Co-ordinated Research Project (CRP) which has just started on antibodies immobilized on magnetic particles. Many problems are creating serious obstacles to the production of these reagents, either in kit or in bulk form, in our countries. These, in our opinion, are primarly: (1) lack of a local technological substrate; (2) great difficulties and costs of importation of the reagents and equipment lacking; (3) research and production concentrated in groups mainly dedicated to basic research; (4) the strong preference of clinical assay laboratories for kits rather than bulk reagent utilization; (5) very limited financial support given to social health programmes by the local governments; (6) non-existence of a local industry interested in RIA and IRMA kit production. The Brazilian experience in this field of activities is also analysed, especially as regards the preparation of tracers, standards and solid phase coupled antibodies for triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) assays. It is interesting to observe that some of the reagents produced by the Brazilian reference laboratory in the ARCAL VIII programme, namely human TSH reference preparation and the magnetic solid phase separation system for human TSH–IRMA, have met with more interest in other countries of the region than in Brazil itself. Some possible solutions are also considered. The final answer is obviously the creation of a locally developed technological substrate of reagents and equipment with the consequent development of industrial kit production. As an immediate solution, a regional collaborative production of in-bulk reagents should be implemented, strengthening the work done by public institutions such as universities, research institutes and hospitals, whose contribution and efforts should be motivated and rewarded with better research facilities. The creation of a regional information and distribution centre and the speeding up of customs procedures is also considered necessary.

1. INTRODUCTION

Our experience in the field of radioimmunoassay (RIA) an immunoradiometric assay (IRMA) reagent production and standardization in developing countries is especially derived from our participation as a reference laboratory in the IAEA organized ARCAL VIII programme (Regional Co-operative Arrangements for Promotion of Nuclear Science and Technology in Latin America) and its related Co-ordinated Research Project (CRP), mainly dedicated to the thyroid related hormones triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) for Latin America, and in the recently started CRP on Antibodies Immobilized on Magnetic Particles for RIA and IRMA of Hormones.

One of the greatest achievements of these programmes is the possibility offered to every participant to be in touch with the reality of many different countries, sometimes similar and sometimes quite different. One of the main problems in developing countries is isolation, not only from the developed countries, but also from each other. Besides providing a very useful interchange of scientific experiences and even products, such programmes can give us an idea of our general situation and problems and help us try to find solutions in a less isolated or, better, in a more collaborative way.

Before entering directly into the analysis of problems, it would be useful to define exactly what we mean when we say "reagents for RIA and IRMA". In our opinion these are the basic and principal constituents of a diagnostic kit, which can also be prepared and distributed in bulk: the tracer (radioiodinated antigen or antibody), the standard preparation and the separation system, commonly an antibody used in liquid phase or coupled to a solid particle. This clarification is useful, because it indicates the two ways in which these diagnostic reagents can be prepared, distributed and used, and it will be seen that the problems and solutions can be different for bulk reagents or kits. From our experience in Latin America and other parts of the world, it appears that kit production must be performed by a specialized industry, leaving aside the question as to whether it has to be a private or state owned manufacturer. What is important, in either case, is that it has to be a group of people especially dedicated to that activity: a highly technological routine with great emphasis on constant quality control and reproducibility. Of course this group of people can dedicate part of their efforts to applied research and development. This activity should not be carried out by institutions whose main objectives are academic, basic research or even clinical work.

The approach can be a little different for bulk reagent production. This can be considered a temporary solution, especially interesting when countries have urgent problems, e.g. in social health care or population screening, or when considering setting up technology to be transferred to a local industry. Such a temporary or emergency task can be carried out very well also by universities, research institutes or

hospitals, if capable and willing to collaborate. The analysis that follows keeps in mind this important distinction in trying to find possible solutions.

2. IDENTIFICATION AND DEFINITION OF PROBLEMS

Besides the known financial limitations and those deriving from suboptimal use of the limited resources sometimes available, there are some other more specific problems that affect, maybe to a different extent, all Latin American and developing countries.

First of all there is the lack of a technological substrate or infrastructure. This affects the local preparation of all sophisticated reagents and equipment. The preparation and utilization of the above mentioned RIA and IRMA reagents require mainly basic products, equipment and accessories that cannot be found inside the country. The importation of this substrate therefore becomes necessary, and all private and public institutions have to face great difficulties such as exorbitant costs, unbelievably long bureaucratic processes, and enormous delays in shipment and customs clearance. These problems probably have historical, cultural and political origins whose analysis and solution are beyond the scope of this paper. These are, however, most important and they are causing a number of problems.

Most of the groups in our countries are doing admirable work in RIA reagent preparation and are composed of research people and clinicians belonging to public and governmental institutions such as universities, research centres or hospitals. Their tasks, vocations and structures are not really oriented to the routine production of reagents and even less to their related marketing and distribution. At the same time these institutions do not usually have (and this is true all over the world!) the administrative and financial flexibility that can partly neutralize some of the primary problems mentioned above, e.g. the enormous difficulties in obtaining or importing reagents and equipment. Many of our countries live with constantly high inflation rates and the utilization of limited funds available is normally slowed down by heavy bureaucratic processes, most of the time with no possibilities of maintaining the purchasing power of the allocated money or of changing its destination. Good laboratories have suffered great delays in a project because they had no money for technical assistance, even with an excess funding for the acquisition of reagents. They had no way to change the 'label' of the money!

When these courageous groups obtain a final good quality product, they have, with neither a marketing organization nor a vocation, to compete with practically all the first world companies acting in the field that are present locally with their sometimes very sophisticated modern kits. In the specific case of Brazil it is really difficult to find a laboratory that is willing to introduce the utilization of in-bulk reagents: even the laboratories of good technical level that could use them normally want the

most sophisticated and expensive kits available that are already in use in the larger private clinical assay laboratories of the country. This is probably also the situation in Argentina and other large countries.

In-bulk reagents could be very usefully prepared for utilization in particularly large screening programmes such as those for neonatal hypothyroidism, phenylketonuria, reproduction hormones, etc., and in some cases they are already being used with great effect and economic advantage. This is one of the achievements of the ARCAL VIII programme in some Latin American countries, but this happens rarely and insufficiently. Here another of the main problems may be identified: the very limited financial support given by the national authorities to dealing with grave social health problems. Where the local government has given priority and funds to some social health programme we also have better established kit or in-bulk reagent production. In other countries, where a state law may even establish that every newborn has to be submitted to screening tests, but the government does very little to support the application of this law, only imported kits are used.

There is then a final problem to consider, related to most of the problems already described: the non-existence of any local private industry that is producing or that is even interested in the production of RIA and IRMA kits. In all Latin America we know of only one case: a Brazilian manufacturer who, with many difficulties, is operating in this way. Again, the reasons are the lack of technological infrastructure, the importation costs and difficulties, the intense marketing carried out by large first world companies and the non-existence of state financed programmes. All these factors together create very high costs, aggravated by the still limited local demands, which obviously makes this type of private enterprise not very attractive. This reality is an obstacle for the whole country and also for those groups producing, or trying to produce, in-bulk reagents. An interested local private industry would stimulate their work, accept the transfer of their technology and could help prepare or obtain some of the basic technologies that are necessary for the development of the final products. It is interesting to observe that this last problem, experienced by all those working in RIA reagent preparation when approaching private managers, and which is a consequence of all the other problems, itself holds the key to the best solution of the general problem. Only a local industry producing good quality kits will provide the final answer. The reason is very simple: this is the way the problem was resolved in the countries whose technological and economic model is now applied throughout the world.

In conclusion, six main obstacles to the production of RIA and IRMA reagents and kits in the developing countries may be identified. These are:

(1) Lack of technological substrate, e.g. the local production of basic reagents, equipment and accessories.
(2) Great difficulties, cost, bureaucracy and delay in the importation of the above mentioned necessary substrate.

(3) Concentration of research, development, production and sometimes also application of RIA reagents in the hands of groups mainly dedicated to basic or clinical research.
(4) Strong aversion of most of the laboratories to the adoption of in-bulk reagents.
(5) Very limited financial support given by the local governments to social health programmes.
(6) Non-existence of a local private industry interested in the production of RIA and IRMA kits.

3. PRACTICAL EXPERIENCE IN BRAZIL

Our laboratory, like any other reference laboratory within the ARCAL VIII project, dedicated most of its activities to the preparation of RIA reagents for T3 and T4 and IRMA reagents for human thyroid stimulating hormone (hTSH). Because of our limited experience in antibody production we did not join in this effort. A collaborating laboratory from Butantã Institute (São Paulo) is now working in this direction, thanks to a Technical Co-operation Project approved by the IAEA. At the same time, being one of the few laboratories in the region with experience in pituitary hormone extraction and purification, we dedicated more efforts to the preparation of an ampouled hTSH standard that we distributed to 20 laboratories in the 14 ARCAL VIII participating countries. Without entering here into technical details (some of the results obtained are presented in other papers in this symposium, i.e. IAEA-SM-324/61, 62, 65, 67) we can say that we carried out the preparation of T3 and T4 tracers and standards, radioiodinated monoclonal anti-hTSH antibody, standard hTSH and polyclonal anti-hTSH antibody coupled to magnetizable cellulose. Together with an international collaborative study carried out on the ampouled hTSH reference preparation we also did a national inter-laboratory study on the assay performance of the three thyroid related hormone radioassays. We had some technical problems, now practically resolved, in the preparation and reproducibility of T3 and T4 standards and we still have some unsatisfactory non-specific bindings related to the utilization of our magnetic solid phase in hTSH-IRMA. As imported materials we are using NETRIA (North East Thames Region Immunoassay Unit, London, United Kingdom), anti-hTSH monoclonal antibody for radioiodination and M-104 magnetizable cellulose particles (Scipac, Sittingbourne, United Kingdom) for coupling: all other materials, including the magnetic separators, are prepared in Brazil.

In conclusion, considering mainly the practical utilization of our in-bulk reagents, we have found a great interest in the region (Latin America) especially for the ampouled hTSH reference preparation and for the anti-hTSH magnetic solid phase. Interestingly enough, this was mainly demonstrated outside Brazil. Of the six

collaborating Brazilian laboratories only one demonstrated an interest in the utilization of in-bulk reagent. Most of them have shown, during the 3–4 years the programme has been running, increasing interest in the utilization of kits, mainly non-radioisotopic, with antibody coated microtitre plates. A neonatal screening programme for hypothyroidism is utilizing immunofluorometric assay in microtitre plates. It started practically simultaneously with ARCAL VIII and is probably, after the Cuban programme, analysing the most newborn in Latin America. One may note the strong tendency towards the utilization of imported kits or reagents in larger countries such as Brazil and Argentina; only one country in Latin America (Cuba) has its own production of kits and is applying them on any scale. There is some interest in bulk reagent production and utilization in smaller countries, especially where there is interest in starting a screening programme using radioisotopic systems.

4. POSSIBLE SOLUTIONS

One problem not mentioned is the 'scientific gap'. Whilst there is a lack of technological substrate, mainly the lack of materials, there is no major backwardness in basic science affecting the technology of reagent production. Even where some gap exists, it is not a significant factor in this field. Nor are more courses, theoretical and academic training needed in this area. Such activities are useful for implementing basic research, and, of course, practical training courses are also very useful, especially for technicians willing and able to use in-bulk reagents. However, such courses will not counter the increasing interest in highly sophisticated imported kits. In many developing countries the scientific and technological capacity for the production of these reagents exists and when this is not sufficient the best thing to do is to send the right person from the right laboratory for an appropriate period of training, not forgetting that, most of the time, adequate training could be offered within the country, or at least within the region. What is really more important is to provide easy and cheap access to the basic reagents and equipment that are necessary for the final RIA or IRMA reagent production, be they imported or locally produced. Each professional, according to his type of work, must have easy access to the basic substrate and structures that are necessary for his task. It is, for example, extremely expensive, dispersive and inefficient to have a good clinician wasting his potential and experience on RIA reagent production: he should have easy access to good kits. At the same time a protein chemist working in RIA reagent production should not necessarily synthesize magnetic iron particles: he should be able to buy them or to have, within the region, a chemical structure providing them. Our laboratory, in order to produce radioiodinated pituitary hormones, years ago started to process human glands. We acquired technology and we are also, in a sense, satisfied with it, but was it really the best way to resolve the problem of RIA reagent production?

These abnormal situations are not only frequent in developing countries, but they are often even encouraged by the local governments or financial entities. But, how can this situation be changed? How does one create a developed technological substrate in situations where the technological gap with the first world is even increasing? The answer cannot ignore educational, social and economic considerations but, whatever it is, it is not a short term task, and in this analysis we are more interested in immediate, working alternatives and solutions. These will not be definitive answers, but in practical terms they can resolve some problems.

One of the answers comes from the previously described experience with ARCAL VIII and with the starting CRP on immobilized antibodies on magnetic particles: use as much as possible the knowledge, the substrate, the materials that are available within the region. There are quite different realities in the different countries and sometimes there are extremely critical situations, with relation, for example, to iodine deficient areas or to the necessity of a screening programme, that have stimulated some national or international bodies to finance the starting of a project that, with limited funding, can only be carried out through the use of in-bulk reagents. At the same time there may be a country that, for some reasons, has developed a certain product or a certain technology that others do not have yet: this has been, for example, the case with some tracers or antibodies, with different types of magnetic particles that can be used for coupling or some hormones produced by classical gland extraction or by DNA-recombinant techniques. Some of these collaborative exchanges and productions are starting to work within the ARCAL VIII programme. If we consider the whole region instead of a single country we can have a useful exchange of reagents and technology at a much better price than if we utilize big first world companies. The laboratory that has a product to offer finds a market, even if limited; the laboratory that needs the product finds maybe the only possibility at the level of its resources. What can be a long distance goal for a single country may become a practically immediate solution and application if we can utilize the collaboration among different laboratories having separately developed different reagents or the technologies that can lead to these reagents. This is a very ancient law, but it can be applicable also to the problem of RIA reagent production. The IAEA-organized programmes which stimulated and started such activities should now encourage their implementation at a production level, starting a collaborative industry, which benefits the products in the following ways:

(1) Possibility of easy exchange of materials among the countries of the region.
(2) Creation of a centralized information and distribution unit.
(3) Possibility, for the producing laboratory, to make a 'profit' that can be directly invested in its own research and development.

Unfortunately, the first point still depends on the previously mentioned problems and delays in customs clearance, a chronic problem that has many times

been unanimously indicated as 'obstacle number one' during ARCAL VIII co-ordination meetings. Perhaps a solution can more easily be found with the help of the IAEA, the United Nations Development Programme (UNDP) and each national government.

Also, the creation of a regionally centralized distribution laboratory has been frequently mentioned at various stages of development of the programme and should be feasible.

Last but not least, experience has taught us that only a modern, flexible and open minded way of converting into better research facilities even limited financial resources derived from their work can motivate good quality research people to this difficult and highly technological routine of reagent production.

Only through the application of these conditions 'sine qua non', an effort that should involve international and national authorities, can we see some possibility of success of this Collaborative Reagent Production: a new CRP.