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**THE RADIOIMMUNOASSAY OF  
HORMONES FOR CLINICAL  
TRIALS OF FERTILITY  
REGULATING AGENTS IN  
DEVELOPING COUNTRIES**

**Report of a WHO Meeting of Experts**

WORLD HEALTH ORGANIZATION

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WHO MEETING OF EXPERTS ON THE RADIOIMMUNOASSAY OF  
HORMONES FOR CLINICAL TRIALS OF FERTILITY REGULATING  
AGENTS IN DEVELOPING COUNTRIES

*Bangkok, Thailand, 13-17 January 1975*

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# **RADIOIMMUNOASSAY OF HORMONES FOR CLINICAL TRIALS OF FERTILITY REGULATING AGENTS IN DEVELOPING COUNTRIES**

**Report of a WHO Meeting of Experts**

## **1. INTRODUCTION**

The last decade has seen the rapid development of research in human reproduction, much of which has dealt with problems of importance to family planning programmes, particularly in the developing countries. Major incentives for this research have been the need to make full use of currently available family planning methods and the necessity of developing new technology in fertility regulation. The introduction of an existing, or a new, method of fertility control in a population requires the establishment of "normal" physiological data for the various ethnic or cultural groups comprising that population, followed by an evaluation of pharmacological and metabolic responses to the proposed methods and the monitoring and elucidation of any side-effects that may occur. In pursuing these objectives it is frequently necessary to set up technical and laboratory facilities in the developing countries. One of the more expensive services in terms of capital and maintenance costs and personnel are laboratories for the assay of hormones associated with reproductive biology.

The following questions arise in connexion with the establishment of these facilities.

Which hormones and other substances require to be estimated?

What analytical techniques are currently available, or may become available in the foreseeable future?

How are assays to be standardized and validated?

Where could these assays most appropriately be carried out?

What laboratory resources (staff, space, equipment, etc.) are needed?

What are the requirements in terms of training, maintenance, and overall costs?

How might conflicting demands for assays for research purposes and for patient care be resolved ?

Can the assay facilities, when established, be used on a regional basis ?

## 2. GENERAL CONSIDERATIONS

The study of the effect of a drug in man is customarily divided into four phases.

Phase 1 is administration for the first time of a therapeutic agent, to human subjects following pharmacological and toxicological studies in animals, which themselves might require hormone assays. The main objective of phase 1 is to ensure that the drug has no gross toxicological effect in man. This phase is not designed to assess therapeutic efficacy but only to study clinical pharmacology, pharmacokinetics, pharmacodynamics, and the hormonal and metabolic responses to the introduction of the new drug in a small group (usually less than 10) of informed volunteers. The duration of the study may be as brief as 4 weeks when a short-acting compound is investigated, but the time required for the study of, for example, an injectable hormone preparation for women designed to be effective for 3 months would be greater, covering a pre-treatment menstrual cycle, detailed observation during the period of activity of the drug, and the time during which all the parameters studied return to their pretreatment levels.

In phase 1 studies there is a demand for frequent sampling and accurate assays of multiple endocrine and other parameters, including serial assay of appropriate gonadal steroids (progesterone, estradiol, and testosterone, for example) and the pituitary gonadotrophins (follicle stimulating hormone (FSH) and luteinizing hormone (LH)). Other assays required would include those necessary for studying changes in hypothalamic-pituitary function such as thyroid hormones, growth hormone, and prolactin levels and the insulin response to a glucose load. Studies on the renin-angiotensin-aldosterone system and on coagulation and fibrinolytic factors may be included. In addition, specific assays of the drug and/or its metabolites are necessary.

Phase 2 study involves up to 50 subjects. In this phase efficacy is studied for the first time. Although fewer endocrine and metabolic investigations are required for each individual subject during phase 2 the total number of specimens for assay may be greater than in phase 1 studies owing to the larger numbers of subjects participating. This

Phase usually lasts from about 3 months to 1 year, and the more common side-effects of the drug may emerge during this period. Additional toxicological studies in animals are also undertaken at this time.

If the phase 2 study gives satisfactory results for efficacy and safety the trial advances to phase 3, which usually involves several thousand subjects in a multicentre trial that may last for 2 years and include careful follow-up of subjects during treatment and after the discontinuation of treatment. The need for assays at this stage is usually minimal, but assays may be required again in intensive studies to elucidate the mechanisms of specific side-effects that might arise.

Phase 4 is the stage of general release and potential widespread use of the drug. Uncommon complications may be seen for the first time, during this phase. Although infrequent, these complications may be potentially serious and include death from thromboembolism following use of an oral contraceptive agent or the effects of interaction between an oral contraceptive and a pathological condition such as disease or malnutrition. When these complications are identified further detailed laboratory investigations have to be made.

In the investigation of infertility, hormone assay has little to offer for the majority of patients, but for the very small proportion of infertile men, the 10% of infertile women who are anovulatory, and the smaller proportion of women who may have corpus luteum insufficiency assays of gonadal and pituitary hormones are necessary for diagnosis. In the monitoring of the treatment of women with gonadotrophins to induce ovulation the assay of gonadal steroids is mandatory.

Assay of estrogens in urine or plasma is of value in monitoring pregnancies in which the fetus is classified as "high risk". In abnormal pregnancy, assay of the  $\beta$  subunit of human chorionic gonadotrophin (HCG) is helpful in diagnosis, and essential for follow-up, of patients with trophoblastic disease such as hydatidiform mole and chorio-carcinoma.

### 3. HORMONES AND OTHER SUBSTANCES REQUIRING ESTIMATION

It is not feasible to list all assays that may be needed in the study of a particular method of fertility control but an outline is given in Table 1 of those considered to be "essential" and those that may be "desirable" for two different groups of agents used for the control of fertility—namely, steroids and prostaglandins.

TABLE 1. "ESSENTIAL" AND "DESIRABLE" ASSAYS FOR THE INVESTIGATION OF STEROIDS AND PROSTAGLANDINS USED FOR FERTILITY CONTROL

Method of fertility control	Essential assays	Desirable assays
A. Steroids	Progesterone, estradiol, testosterone, cortisol, follicle stimulating hormone (FSH), luteinizing hormone (LH), therapeutic steroid and some metabolites	Triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH), insulin, prolactin, human growth hormone (HGH), components of coagulation and fibrinolytic systems, renin-angiotensin-aldosterone
B. Prostaglandins (PG)	PG and stable metabolites and the assays listed above where relevant	

The need for these assays can be summarized as follows. While progesterone assays alone may provide indirect evidence of the presence or absence of a functional corpus luteum, measurement of estradiol is required to assess the development of follicular function and thereby indicate the degree of ovarian suppression. Assay of pituitary gonadotrophins is required to determine whether there is merely interference with the mid-cycle peak of luteinizing hormone or total suppression of gonadotrophin secretion. Assay of testosterone will be needed to determine whether male physiological function is impaired by any drug used for fertility control in men. Cortisol should be assayed because therapeutic steroids may interfere with the secretion, transport, binding, or biological activity of this hormone. Regarding the prostaglandins, since the naturally occurring substances are so transient in biological fluids it is necessary to develop a radioimmunoassay for a stable metabolite that will give a clear idea of the kinetics of action of the administered compound.

The assays considered to be desirable are particularly related to body systems that may have been secondarily affected by the method of fertility control employed. Because many steroidal agents induce changes in circulating proteins that bind hormones it is necessary to determine whether triiodothyronine, thyroxine, and cortisol in the biologically active state have been affected. In view of the known effect that some of the existing steroidal contraceptives have on carbohydrate metabolism it is also necessary to follow changes in carbohydrate tolerance. These changes are monitored most sensitively by serial glucose tolerance tests using as a parameter the insulin response to administered glucose as well as the levels of glucose in serum or plasma.

The need for prolactin assay is indicated by the results of toxicological studies in animals. It is known that some of the agents currently used for contraception cause an increase in prolactin secretion in the dog or rat, which may be associated with the induction of breast neoplasia. Galactorrhoea may occur in some patients being treated with certain steroids, and with some methods of fertility regulation there may be interference with physiological lactation. Therefore, circulating basal levels of prolactin should be assayed and changes in level in response to stimulation or suppression should be studied. Again, because of the known effects of depot medroxyprogesterone acetate on the production of growth hormone there may be a need to study the effect of other therapeutic agents on this hormone.

Since thromboembolism and hypertension may occur as side-effects resulting from the use of existing steroidal contraceptives assessment should be made of appropriate measurements of changes in the components of the coagulation and fibrinolytic systems or the renin-angiotensin-aldosterone system.

So far, little reference has been made to the need for methods of measuring therapeutic substances in biological tissues and fluids. In order to study the pharmacokinetics of any new compound it is essential to develop an assay method for the substance itself and if possible for its metabolites. Only one or two laboratories, which would receive samples of plasma for assay, would be required to make these tests. This is a good example of the need for regional, or even international, cooperation in the development and use of assay techniques. The setting up of specific assay facilities for a new therapeutic substance that may never enter clinical use cannot be justified for individual assay laboratories.

Almost all of these assays will be carried out using blood plasma or serum samples but some tests on other body fluids such as milk or seminal fluid may be needed. To assess tissue exposure to biologically active cortisol the measurement of urinary free cortisol in 24-hour urine specimens is still the simplest method at the present time.

It must be stressed that the list of assays given in Table 1 is not exhaustive. Additional assays will undoubtedly be required for the investigation of some therapeutic substances.

#### 4. CURRENTLY AVAILABLE ANALYTICAL TECHNIQUES AND TECHNIQUES THAT MAY BE AVAILABLE IN THE FORESEEABLE FUTURE

A wide variety of analytical techniques applicable to the assay of hormones and other compounds have been developed. These can be classified into three categories.

(a) *Physical and/or chemical assays.* This category includes assays based on physical and/or chemical methods such as colorimetry, fluorimetry, isotope dilution techniques, administration of isotopically labelled compounds and measurement of isotope concentrations after fractionation of blood samples, gas-liquid chromatography, and mass spectrometry.

(b) *Biological assays.* Assays based on the response of the target organ to varying doses of the compound to be assayed either *in vivo* (e.g., assay of LH by measuring the change in ovarian ascorbic acid concentration in rats) or *in vitro* (e.g., assay of LH by cytochemical techniques).

(c) *Protein binding assays.* These include assays based on the non-covalent binding by a protein of the compound to be measured. In most cases the relative amounts of the compound bound to the protein or present in the free state are determined after the addition of a small amount of the labelled compound. The specific protein may be an antibody (immunoassay), a tissue protein (receptor binding assay), or a circulating protein (circulating protein binding assay).

For many compounds, particularly protein and polypeptide hormones, estimates obtained with the same sample will differ according to the assay method employed. Therefore, the method and standard preparation used must always be stated, in accordance with the recommendations of the twenty-sixth WHO Expert Committee on Biological Standardization.<sup>a</sup> Results of steroid estimations should be expressed in the *Système International d'Unités* (SI units).

In research on methods of fertility control, assay of large numbers of samples will be necessary. The assay methods used should be simple to perform, capable of automation, inexpensive, have satisfactory reliability criteria (specificity, sensitivity, accuracy, and precision), and be free from significant health hazard (radiation, toxicity). Many of the

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<sup>a</sup> WHO Technical Report Series, No. 565, 1975.

techniques listed above have disadvantages in respect of technical difficulty, low throughput, and high capital cost and running expenses. Radioimmunoassay satisfies most of the requirements and is, in the opinion of the Meeting, the method of choice at the present time.

Radioimmunoassay has, however, a number of disadvantages including the high cost and general non-availability of radioisotope counters; the difficulty of developing fully automated systems; a slight health hazard (arising from the preparation and use of labelled antigen); and the relatively short shelf-life of compounds labelled with isotopes emitting  $\gamma$ -radiation. Alternative analytical procedures are therefore being developed that take full advantage of the specificity and sensitivity associated with the use of antibody but do not employ a radioactive label.

To compete successfully with radioimmunoassay alternative methods must be equally sensitive and precise and the detection system must be as simple and efficient as possible. Three alternatives suggest themselves—namely, (1) labelling the antigen with a non-radioactive compound such as an enzyme; (2) the use of specific antibodies labelled with a non-radioactive compound; and (3) techniques that do not involve the labelling of either reactant. Examples of techniques in the third category are fully automated nephelometric assays, which are based on the scattering of light by antigen-antibody aggregates. The sensitivity of these assays is, however, limited to the nmol/litre range and the technique is not applicable to steroids and other haptens. A recent modification of this approach, in which unreacted antibody is used to aggregate a fixed amount of antigen covalently linked to a carrier protein, can determine 10 ng, or less, of progesterone. However, problems arise when this method is used in the assay of biological fluids.

Enzymes, fluorescent compounds, and bacterial phages have been employed for labelling as alternatives to radioisotopes. For example, enzyme-labelled immunoassays, which depend on conjugating an enzyme to the antigen without impairing either the enzymic activity or antigen-antibody binding, have been developed for a number of hormones including estradiol, estriol, and HCG. Certain of these assays offer the major advantage that they do not require separation of the bound and free fractions. However, the steric hindrance between the enzyme and its substrate, associated with antibody binding, may block the enzymic activity in the bound fraction.

Despite intense research activity in the fields of non-isotopic immunoassay and other assay procedures, it seems unlikely that radioimmunoassay will be replaced as the analytical method of choice in the evaluation of methods of fertility regulation during the next 5 years.

## 5. STANDARDIZATION AND VALIDATION OF HORMONE ASSAYS

In setting up hormone assays one of the main problems encountered is the difficulty of obtaining suitably characterized reagents that produce accurate and reproducible results.

The need for standardization in immunoassays is part of the wider problem of standardization in diagnostic assay methodology on which the twenty-sixth WHO Expert Committee on Biological Standardization has recently reported.<sup>a</sup> The Committee's report contains recommendations for the assessment of assay systems, and assays used in research programmes in reproduction, such as collaborative trials of fertility regulating agents, should comply with these recommendations wherever possible.

While it might at first sight appear convenient for laboratories engaged in such research programmes to purchase or try to make their own assay reagents, the Meeting strongly recommended that a central supply of matched, characterized reagents should be made available for these laboratories.

Adoption of this recommendation would lead to the following important advantages: improvement in the comparability of assay results; avoidance of the effort and delay involved when laboratories attempt to make and standardize their own reagents, a process which would take at least 6 months; considerable saving of time in establishing assays; and substantial reduction in costs.

The reagents that should be provided to carry out the hormone assays regarded as essential (see Table 1), include:

(a) *Standards.* For peptide hormones the appropriate WHO international reference preparations should be used.<sup>a</sup> If adequate supplies of these preparations are not available a suitable working standard should be made and calibrated in terms of international units. In the case of chemicals that are available in pure form (steroids and prostaglandins, for example), preparations of the highest purity are required.

(b) *Ligand for labelling.* A quantity of a highly purified, well characterized preparation of each peptide hormone is required. To ensure their stability, convenience of handling, and economical use these materials could be distributed in microgram amounts in glass capillaries suitable for isotope labelling. Isotopically labelled chemicals (steroids

<sup>a</sup> WHO Technical Report Series, No. 565, 1975.

and prostaglandins) and isotopes such as free  $^{131}\text{I}$  and  $^{125}\text{I}$  for labelling the peptide hormones could be obtained by each laboratory from commercial sources.

(c) *Antisera.* For each hormone an adequate supply of selected antiserum in a stable (preferably freeze-dried) form should be made available in ampoules.

(d) *Reagents used for the separation of bound and free hormones.* Preferably, "second antiserum" should also be provided, although it would be difficult to obtain the amount that might be needed as a single batch.

The peptide ligands for labelling, the specific antisera, and any additional working standards that may be necessary should, if possible, be freeze-dried and placed in ampoules under the same conditions as those used for the preparation of international biological standards.<sup>a</sup> These reagents should then be assessed, both individually and as matched reagents, by at least two independent laboratories.

The continuous monitoring and assessment of assay systems to ensure reproducibility and comparability of results is another major problem in radioimmunoassay. This area is neglected in many laboratories in the developed as well as the developing countries. Following the establishment of an assay it is essential that assay stability and continued reliability should be carefully monitored. A quality control programme for radioimmunoassay should cover three aspects of monitoring: (1) evaluation of the standard curve and assay error; (2) characterization of antibody-antigen binding; and (3) evaluation of the assay result of standard specimens. Coded unknown specimens from an external sample should be included at regular intervals and the data should be analysed by the centre responsible for the external quality control scheme.

Continual updating of control parameters can most conveniently be achieved through fully computerized data analysis and quality control programmes. However, in many laboratories where major computer facilities are not available most of the quality control parameters required can be analysed by means of laboratory-based, programmable, desk-top calculators. The graphic display of quality control parameters plotted over time can be helpful in evaluation of the performance of the current assay as compared with that of previous assays.

Whether or not computer (major or desk-top) facilities are available, control charts should be maintained by plotting the results obtained for

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<sup>a</sup> Unpublished working document WHO/BS/773.65.

known specimens, which should be included in each assay batch. Although it is useful to ascertain statistical limits of variance such as  $\pm 2$  standard deviations of the mean it is more important to watch for persistent trends and sudden alterations in variance. A greater ability to analyse such changes is achieved by the use of cumulative sum ("cusum") techniques, which present the information necessary for assessing the distribution of results and drifts and other changes in the distribution in a manner allowing of easier interpretation.

In addition, a logbook should be maintained for each analytical method in which records are kept of changes in personnel operating the method, reagents, standards, instrumentation (e.g., breakdowns and repairs), and major variations in ambient temperature and humidity.

The Meeting recommended that a group of appropriate experts be convened to implement a programme for the selection, preparation, and distribution of sets of matched reagents for hormone assays. This programme could then be responsible for:

(1) the provision, characterization, ampouling, and quality control of matched reagents according to the criteria established<sup>a</sup> and detailed information about the reagents and manner of performing assays, perhaps in the form of a manual of protocols;

(2) making recommendations on arrangements for the monitoring of hormone assay performance by laboratories engaged in research in human reproduction and the organization of an external quality control scheme through the distribution of coded samples for testing and the coordination of results;

(3) the facilitation and integration of collections of human pituitary and other tissues for the extraction and preparation of purified hormones and other antigens at the national and the international level.

Protocols should also include instructions for obtaining and storing samples of body fluids. If it is necessary to send samples for assay to laboratories located at a distance, those samples in which the hormones are unstable should be maintained at temperatures below  $-40^{\circ}\text{C}$  or, preferably, be freeze-dried. Laboratories may therefore need to be provided with freeze-drying facilities.

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<sup>a</sup> WHO Technical Report Series, No. 565, 1975.

## 6. ESTABLISHMENT OF ASSAY FACILITIES

Since circumstances will vary in different countries the criteria used for deciding on the location of a hormone assay laboratory engaging in the clinical trial of fertility regulating agents should be flexible to some extent. However, the Meeting considered the following criteria worthy of consideration.

(1) An assay centre should be established in a locality where there is a tradition of research and known competence in carrying out clinical trials.

(2) The centre should be associated with clinical facilities and be located within, or adjacent to, a large hospital with a family planning clinic. The centre should preferably be affiliated to an academic department of obstetrics and gynaecology; this arrangement would allow the clinical director, the laboratory head, and other members of the staff of the centre to have appropriate academic status.

(3) The centre should be located in the vicinity of a clinical chemistry laboratory and have access to a library and to chemical, statistical, and other specialized supporting facilities. Whenever possible the assay centre should be based on an existing centre with experience in radio-immunoassay and related techniques.

(4) An adequate number of personnel with a scientific background should be available to staff the new laboratory or should be trained for this purpose. The person appointed as head of the laboratory must be willing to devote all his efforts to establishing and running the laboratory, and his professional status must be such as to ensure the support of his clinical and scientific colleagues.

(5) Adequate space should be available to house the laboratory.

Several members of the Meeting have had personal experience in the problems involved in establishing a new laboratory in a developing country. These problems include the difficulty of attracting a suitable person to head the laboratory; the difficulty of retaining staff once they have been fully trained, mainly owing to financial considerations; a shortage of personnel familiar with immunoassay; and difficulties with equipment maintenance and both electricity and water supplies.

Recommendations for at least the partial solution of these problems are made in appropriate sections of this report.

## 7. REQUIREMENTS FOR A HORMONE ASSAY LABORATORY

### 7.1 Personnel

The scientific personnel for a hormone assay laboratory as envisaged here should comprise, in addition to the head of the laboratory, at least two graduates for steroid assays and two for peptide assays together with appropriate ancillary staff (assistants to wash glassware, for example).

The head of the laboratory is, like the clinical director designated to direct the clinical aspects of the work, a key appointment. The person selected should preferably have had some previous experience in hormone assay methodology, his standing in the scientific community should be high, and he should have demonstrated his competence in scientific research. To facilitate the interpretation of hormone assay results for clinicians it is desirable that the laboratory head should be medically qualified or hold a degree in the biological sciences and have had substantial experience in clinical pathology. If the person appointed has had little experience in radioimmunoassay he should receive suitable training in this subject, as discussed in the next section.

The head of the laboratory should be given a full time appointment and be involved in the planning of the laboratory from its earliest stages. He should be prepared to remain in the post for a reasonable period. Considerable importance is attached to the security of tenure of staff appointments.

### 7.2 Laboratory space

To accommodate the personnel and equipment listed above approximately 30 m<sup>2</sup> of working space should be provided for each of the steroid and the polypeptide/glycoprotein hormone laboratories. In addition, space in the total laboratory area should be provided for ancillary functions (e.g., an instrument room, an office, and a storeroom), bringing the total requirement to about 100 m<sup>2</sup>. The laboratory space should be divided into two separate areas, one for work with steroids and isotopes emitting  $\beta$ -radiation, the other for work with peptides and isotopes emitting  $\gamma$ -radiation. A fume-cupboard with bench space and adequate ventilation should be incorporated in one of these laboratories and another in a small separate radiochemical laboratory designed for handling quantities of isotope having an activity of 1 mCi or more and used for radio-iodination procedures. This laboratory should be provided with adequate radiation shielding (by means of lead bricks, for example)

and comply with the appropriate international and national regulations relating to protection against radiation hazards. The main laboratories and ancillary rooms should preferably be situated on the same floor of the building. Since radiation health regulations forbid the consumption of food and beverages in radioisotope laboratories, facilities for eating should be provided elsewhere.

Floors should be reinforced to support the weight of several large, heavy instruments (automatic radiation counters, refrigerated centrifuge, etc.) and doors should be wide enough to admit these instruments. It is desirable that a heavy duty lift should be available. Since the instruments generate a considerable amount of heat and noise adequate ventilation is essential and some acoustic insulation desirable. Counters for  $\beta$ - and  $\gamma$ -radiation should be placed far enough apart to prevent any background interference of the  $\beta$ -counter by  $\gamma$ -radiation. Adequate supplies of water and a stable mains electricity supply are needed. If the electricity supply is not very reliable a stand-by generator may be necessary. Regulations concerning safety in laboratories, fire precautions, and requirements for laboratories using radioisotopes (nonabsorbant floors, disposal facilities for radioactive wastes, etc.), as well as considerations relating to specimen handling, should be kept in mind.

In view of the likelihood of increased demand for assays and the extension of the methods to measurement of other substances of importance in clinical medicine, the need for expansion should be anticipated and an additional amount of space, equal to at least 50% of the total originally estimated, should be provided. Further space would be required if the laboratory undertook additional commitments such as serving as a national centre or national control laboratory (see section 7.5).

### 7.3 Equipment

The minimum equipment essential for a hormone assay laboratory is shown in the following list.

<i>Heavy equipment</i>	<i>Approximate cost in 1975 (US \$)</i>
1 liquid scintillation spectrometer with automatic sample changer and data print-out	25 000 <sup>a</sup>
1 $\gamma$ -radiation counter with automatic sample changer and data print-out	22 000
1 refrigerated centrifuge with multiple sample head	4 000

<sup>a</sup> Use of steroids labelled with  $\gamma$ -radiation emitters, which are becoming available, would avoid the need to purchase a separate  $\beta$ -radiation counter, thereby saving US \$25 000 in capital outlay and achieving lower running costs. However, antisera compatible with such labelled steroids would have to be used.

	<i>Approximate cost in 1975 (US \$)</i>
2 deep-freeze units, preferably cooling to $-40^{\circ}\text{C}$ or below, with breakdown alarm system	3 000
2 refrigerators cooling to $+4^{\circ}\text{C}$	500
1 drying oven	500
1 high-temperature furnace	500
1 freeze-drier	3 000
<i>Light equipment</i>	
1 desk-top programmable computer with print-out facilities or	5 000
1 teletype terminal connected to a larger computer	2 500
1 water deionization and distillation unit	5 000
1 micro-analytical balance	3 000
1 analytical balance	500
1 pH meter	500
2 ice makers	2 000
2 vacuum pumps	500
2 bench centrifuges	500
2 manual radiation counters <sup>a</sup>	1 500
2 automatic sample-diluters and -pipetters	2 500
Reusable glassware	2 000
Chemicals	2 000
A standby electricity generator (optional item)	—
<i>Ancillary equipment</i>	
Electrical and mechanical maintenance equipment	1 000
An adequate supply of spare parts for the scientific equipment	10 000 <sup>b</sup>
Miscellaneous equipment and spares	3 000
<b>TOTAL</b>	<b>100 000 <sup>c</sup></b>

The selection of equipment for the laboratory will be determined by the maintenance facilities available in the area. Proof that equipment can be serviced locally must be obtained before purchase. It is advisable to establish equipment maintenance contracts whenever possible, but an adequate supply of recommended spare parts for all major equipment for use in the event of electronic or mechanical failure should be obtained from the manufacturer. It is advisable to maintain automatic counters under air-conditioning.

There may be occasions when reasonable servicing cannot be obtained for the equipment, and the following points must be considered.

<sup>a</sup> These items should if possible be identical so that parts can be interchanged in the event of breakdown.

<sup>b</sup> Approximately 10% of the total cost of equipment.

<sup>c</sup> In addition, 20% of the total should be added for shipping and insurance costs. Also, duty and customs charges will probably have to be included.

(1) Access should be sought to an electronics workshop (possibly within a university physics or engineering department) with experienced personnel. If assistance is to be obtained in this way service manuals and circuit diagrams, as well as adequate spare parts, are essential.

(2) If no relationship of this kind can be established, it will be necessary to set up an electronics workshop in association with the developing laboratory.

The degree of automation necessary in any laboratory is dependent upon the number of assays performed and the rate at which they are carried out. For small numbers of samples, and when there is a need for rapid assay of a few individual samples, the pipetting and similar procedures can be performed manually. In most situations, however, some automated handling may be useful and a wide range of equipment of different degrees of complexity is available. However, in some countries where problems of equipment maintenance occur the use of very complex systems may not be feasible or even desirable.

For analysing results, a small desk-top computer, preferably with data print-out, is generally adequate. If convenient, direct, and reliable access to a larger computer is available, use should be made of the facilities offered.

#### **7.4 Consultant assistance**

Consultant assistance will often be essential in establishing an assay laboratory. If such assistance is considered necessary the consultant should participate from the inception of the laboratory and advise on the choice and purchase of equipment (see section 7.3) and the phasing of inputs such as equipment, staff, and training (see section 7.6). It may be advantageous to recruit two consultants—one to assist in the establishment of the steroid laboratory, the other in the establishment of the polypeptide/glycoprotein hormone laboratory.

Affiliation might be established with a recognized assay laboratory that could provide appropriate consultant expertise and undertake specific training programmes for the new laboratory. Such liaison should continue during the period of development (3–5 years) of the new laboratory.

#### **7.5 Training**

Training may be required for the graduate research assistant and/or technical officers who will carry out the assays. Although it is desirable for these personnel to have had some previous laboratory experience they may be recent graduates.

Training should be given primarily "at the bench", where experience in using assay methods is acquired, but in addition should include instruction in the basic principles of radioisotopic analytical methods; general concepts of immunology; preparation of immunogens; production of antisera; characterization of antisera; isotopic labelling of antigens; separation methods; standardization of reagents; assay design and validation; quality control; data processing; automation; familiarization with equipment; new developments in assay techniques; and laboratory organization. Some instruction should be given in steroid and protein biochemistry and general reproductive biology.

Training can be provided in two ways:

(1) *At the new laboratory* training can be given by appropriately experienced senior scientific personnel already employed at the laboratory or by an outside consultant. The teachers should have had considerable experience in hormone assay, particularly radioimmunoassay, and in laboratory organization and management. It may be necessary for the consultant to spend at least 1 year, and possibly 2 years, at the new laboratory assisting in the implementation of the training programme and the establishment of the assays.

(2) *At another laboratory* training can be given by experienced personnel capable of undertaking a training programme to which personnel from the new laboratory could be sent for periods of 1-2 years.

The Meeting stressed the importance of the in-service aspects of such training programmes and, except for informing experienced workers about new developments in assay methods and techniques, saw little benefit in organizing courses lasting 1 or 2 weeks.

Training should be oriented towards establishing the "essential" assays (see Table 1) for the laboratory (i.e., progesterone, oestradiol, cortisol, LH, and FSH). From the experience gained with these assays it should then be possible to establish the "desirable" assays (Table 1) at a later stage. Should particular problems arise or should it become necessary to set up more difficult assays, technically specialized consultants may be recruited for short periods to visit the new laboratory to advise or to provide additional training.

If the scientist designated as head of the laboratory is inexperienced in radioimmunoassay techniques he should, before the new laboratory is set up, spend up to 12 months training in an active assay laboratory, and during this time he should visit other specialized laboratories.

## 7.6 Phasing of "inputs"

A scheme for the phasing of "inputs" into the new laboratory is outlined in Table 2. It is essential that a timetable should be prepared at the outset and that every effort should be made to adhere to it.

The scheme shown in Table 2 makes certain assumptions that could strongly influence the time schedule. Some of these assumptions are that funding has been allocated and is available, appropriate staff can be found, and all new building work or necessary structural alterations to existing buildings have been completed.

Following the appointment of a clinical director and a head of the laboratory, a meeting should be convened with the consultant or consultants who might be involved in the establishment of the laboratory. The meeting should specify the equipment and supplies required, suggest an appropriate training programme for the personnel of the laboratory, and indicate the preliminary research programme to be established. The research protocols could then be developed and equipment and supplies ordered.

The scheme outlined in Table 2 shows the phasing of recruitment of laboratory staff, possible assistance by consultants; staff training; and the establishment of assays for staff trained either at the new laboratory or at another laboratory, the main difference being, that the training of personnel in the new laboratory cannot begin until equipment is delivered.

## 7.7 Costs and funding

Sources of funding will vary from country to country. In some cases the total costs are borne by the country establishing the laboratory but in others an international agency may provide for the major part of the initial costs. Costs can be broken down into the following components.



(a) *Equipment and supplies.* The capital equipment (see section 7.3) and supplies will cost approximately US \$100 000. Developing countries might obtain financial assistance for these purchases from international agencies since shortages of convertible currency may present difficulties in obtaining supplies from abroad.

It must be remembered that all laboratory equipment has a finite life span and that provision must be made for replacing expensive items of equipment such as automatic radiation counters after 5-8 years of use.

(b) *Running costs.* These include salaries, purchase of supplies and small items of equipment available locally, and laboratory overheads

TABLE 2. SCHEME FOR THE PHASING OF INPUTS INTO A NEW ASSAY LABORATORY

Action	Year 1	Year 2	Year 3	Year 4
1. Appointment of clinical director and head of laboratory	(1 month)			
2. Meeting of head of laboratory and consultant(s) to discuss equipment and supplies, training, and research proposals	(1 month)			
3. Equipment and supplies	(2 months)			
(a) Completion of list of equipment and suppliers	(4 months)			
(b) Ordering, purchase, and delivery of equipment	(1 year)			
4. Production and review of research protocols				
5. Establishment of the laboratory				
(a) Training personnel at the new laboratory	(11 months)	(1 year 6 months)		
(1) Consultant assistance:				
(i) in initial advisory capacity		(2 months)		
(ii) on-site		(1 year 6 months)		
(2) Identification and recruitment of personnel				
(3) Training of laboratory personnel				
(4) Establishment of assays:				
(i) essential		(1 year)		
(ii) additional			(1 year+)	
(b) Training personnel at another laboratory				
(1) Consultant assistance:				
(i) in initial advisory capacity and in training at other laboratories	(1 year 3 months)	(8 months)		
(ii) on-site				
(2) Identification and recruitment of personnel	(2 months)			
(3) Training of laboratory personnel	(1 year 2 months)			
(4) Establishment of assays:				
(i) essential		(8 months)		
(ii) additional			(1 year+)	

 Recommended period of input  
 Possible extension of input

such as the cost of water and electricity and building maintenance. These costs will vary between countries.

(c) *Training and consultant assistance.* Funds should be available to pay for consultant assistance and training. Where some initial costs are borne by an international agency arrangements should be made at the outset to obtain funds from local sources for running the laboratory at a later stage since international assistance is seldom provided on a long-term basis.

### 8. PROVISION OF HORMONE ASSAY SERVICES ON A LOCAL, REGIONAL, AND NATIONAL BASIS

A laboratory totally committed to research on the development and use of fertility regulating agents cannot function effectively without patient material; the active cooperation of clinicians must therefore be obtained. Excess laboratory capacity may be available that could be used, at the discretion of the laboratory head, for clinical tests. This use of spare capacity will increase the awareness of clinicians that the assay laboratory can offer opportunities for the improvement of patient care.

TABLE 3. CAPACITY OF VARIOUS HORMONE RADIOIMMUNOASSAY METHODS

Substance	Method	No. of samples assayed per week
Progesterone	Serum/plasma	200
Estradiol	Serum/plasma	100
Testosterone	Serum/plasma	100
Cortisol (free)	Urine	200
Luteinizing hormone	Serum/plasma	200
Follicle stimulating hormone	Serum/plasma	200
Insulin	Serum/plasma	200
Growth hormone	Serum/plasma	200
Thyroid stimulating hormone	Serum/plasma	200
Prolactin	Serum/plasma	200

With the equipment listed in this report (see section 7.3) the laboratory should have the average weekly capacity for assays shown in Table 3. This does not take account of the use of automated equipment, which would increase the throughput considerably.

For difficult or rarely performed assays regionalization will often prove advantageous. Certain new hormone laboratories might develop expertise in a specific assay and undertake these assays for the country or region.

This applies particularly to the measurement of therapeutic substances in biological tissues and fluids (see p. 9), in which field international cooperation is desirable.

Once it becomes known that a hormone laboratory is providing assay services, the demand for assays is likely to increase, both locally and in other parts of the country. The question then arises whether this service could be shared or integrated with the work of other laboratories, and, if so, how this might be done.

The Meeting recognized that in many developing countries it will not be possible for many years to set up hormone assay laboratories on a scale that would provide a national assay service. Nevertheless, the Meeting considered that, as the health services are extended, a country could introduce a network of hormone assay laboratories in the form of a multi-tier system that initially would operate on a fairly limited scale. Proposals for a national assay service as discussed by the twenty-sixth WHO Expert Committee on Biological Standardization<sup>a</sup> could serve as a guideline for any country to follow. A national assay service of this kind provides an efficient service to the medical community, improves the reliability of the results of the assays through standardization, and, most important, has proved to be notably cost effective in developed countries.

The type of laboratory involved in research in fertility control described in this report could provide the initial expertise in immunoassay methods in a developing country. Through an extension of its own programme, or by its ability to train other workers, such a laboratory could assist in the creation of a national hormone assay service.

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<sup>a</sup> WHO Technical Report Series, No. 565, 1975.