



REQUIREMENTS FOR KITS FOR IMMUNOASSAY AND OTHER PROTEIN BINDING SYSTEMS

Immunoassay - Terms

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INTRODUCTION

The use of immunoassay kits for the estimation of a wide variety of analytes in body fluids is now well established and being used on a vast scale. Many manufacturers and individual laboratories are producing kits which make a very significant contribution to health care throughout the world. Nevertheless, it is possible that kits exist which do not give reliable and uniform results. Several national and international bodies, including organizations in individual and groups of countries, are now preparing independent specifications for the regulation of diagnostic kits.

It is recognized that the problems that could arise from diversities in such regulations could be avoided by making available a set of international requirements and guidelines for kits and their components. An earlier set of recommended criteria for the assessment of radioimmunoassay reagents was made in the 26th Report of the ECBS (1975);¹ the present Requirements are provided in replacement of that document. These Requirements and Guidelines should apply to all kits used in health care, whether they are assembled by manufacturers, or by national or individual laboratories. It is emphasized, however, that although the quality and quantity of each component plays an integral part in the performance of the kit, the usefulness of a component can be assessed only by measuring the performance of the whole kit when it is used according to the manufacturer's recommended instructions.

The assembly of kits is a complex procedure demanding considerable experience, expertise and financial investment. Any national or international body or individual laboratory contemplating the establishment of an organization to assemble kits should carry out a careful cost benefit analysis to determine whether the expertise is available and the investment is justified. An important consideration in such analysis is the variability in the results that may occur within or between batches or lots. In any event, the results obtained with such kits should correlate with those obtained with validated kits and other methods, on the basis of which analyte concentration ranges considered normal or abnormal have been proposed for that body fluid. In clinical practice, the safety of the patient and the effectiveness of treatment may well depend as much on an assurance of the quality of a kit and its method of use as on the quality and quantity of a drug used in treatment.

GENERAL CONSIDERATIONS

An assay kit is defined as a set of reagents and materials intended for the estimation in vitro of a specified analyte to a stated degree of precision when used according to the manufacturer's recommended instructions. The purpose of these Requirements is to help to ensure that sufficient and appropriate information is supplied to enable a national control authority to decide whether a kit is suitable for the purpose stated by the manufacturer.

To make an adequate assessment of each kit or kit component the national control authority should request and consider the data indicated in these requirements and guidelines, in order to determine the extent to which they have been followed. Even after adequate assessment and the release of the product by the national control authority, the decision as to the acceptability of the kit for a particular purpose is the responsibility of the user, who should base his decision on the actual performance of the kit for his required purpose.

There should be available from the manufacturer sufficient information to enable assessment of the performance of their kits to be made according to the criteria recommended in Section . Such information could also facilitate the comparison of products from different manufacturers. It is advisable also that the national control authority should ascertain the extent of routine quality assurance exercised by the manufacturer.

¹ WHO Technical Report Series No. 565, 1975, p. 29

A kit should be evaluated as a whole when carried out according to the manufacturer's instructions and not solely on the basis of criteria for individual reagents. Since the properties of the reagents used for assay of a particular analyte should be related to each other, it is the interrelated quality of the reagents when they are used according to instructions that should be such as to provide acceptable performance characteristics in regard to precision and specificity (Section C).

The specificity of the assay system is its ability to estimate solely the type and kind of analyte it is intended to measure. It should therefore be determined for the particular molecular form of the analyte and nature of test specimen it is intended to assay, and the results of these determinations should be expressed either in quantitative terms or in yes/no test systems. Consequently, for each analyte assay system there is a need for appropriately characterized reference preparations such that the specificity of a kit may be checked and to ensure that the estimates are not affected by other substances that may be present in the test samples. For example, the reference materials for peptide hormones, may consist of highly purified samples of various intact hormones and pure preparations of various related materials, such as the hormone precursor or a subunit;¹ for analytes available in pure chemical form such as steroids and drugs, samples of various other known chemically similar related substances would be used; for hepatitis B surface antigen (HBsAg) a panel of sera for the hepatitis antigen subtypes would be used (International Reference Reagents consisting of a panel of such sera were established in 1978, see 30th Report of the ECBS),² for tumour marker substances, various normal serum proteins, normal cell constituents and related substances from neoplasia of various types would be used. The national control authority should supply or approve of such reference materials used for the assessment of specificity.

Calibration of kits for the estimation of different analytes presents different individual problems for each analyte. Kits intended for the assay of pure chemicals such as steroids and certain drugs should be calibrated in terms of samples of the chemical in the purest attested form available. Kits intended for the assay of peptides, proteins and other biological substances that cannot be characterized entirely by chemical and physical means alone should be calibrated in terms of suitable attested reference biological materials such as the appropriate International Reference Preparation established for this purpose. National control authorities should supply or approve of reference materials used for the calibration of kits. Guidelines for the preparation, handling and characterization of such reference materials are available (Annex 4, 29th Report of the ECBS.)³

Many and diverse methods exist for the statistical calculation of assay results. This poses a problem only when significantly dissimilar estimates can be shown to depend upon the statistical procedures used to calculate them. In any treatment of data, it is preferable that curve fittings be done by an objective statistical method with validated computerized data processing.

Attention is drawn to certain recommendations of the 21st meeting of the Expert Committee on Biological Standardization concerning the manner of expressing the results of immunoassays of hormones.¹ When the results of such assays are given, there should be added the qualification "by immunoassay"; in addition, if the results are given in units, it is essential that the reference material on which the unit was based should also be stated. In all cases, if any preparation (e.g. an international or other widely recognized reference material) has been used to calibrate the reference material in the assay system, this should be identified. Problems that may arise in the calibration or replacement of reference materials in different assay systems are considered in those guidelines (Section 1.4).⁴

¹ Report of the 21st meeting of ECBS, WHO Technical Report Series No. 413, 1969, pp. 7-10.

² WHO Technical Report Series No. 638, 1979, p. 29

³ WHO " " " No. 626, 1978, p. 101

⁴ WHO " " " No. 565. 1975. p. 45-48

When results are given in terms of mass concentration or substance concentration, the Systè^me International¹ should be employed. For ease of comprehension in laboratories where the Systè^me International is not used, appropriate conversion factors should be stated, but the use of SI units should be stressed.

While normally there is a negligible radiation hazard involved in the use of radio-immunoassay kits, all work with radioactive materials should be carried out in conformity with national legislation and accepted codes of practices for protection against radiation hazards.²

Recommended precautions in dealing with potentially infective materials are in preparation by the WHO Secretariat. Such guidelines include directions for proper disposal of waste material that might constitute a public health hazard.

A. REQUIREMENTS FOR THE MANUFACTURE AND ASSEMBLY OF IMMUNOASSAY KITS AND KIT COMPONENTS

A kit shall comprise a number of matched components, including the tracer labelled ligand (or tracer ligand), the binding protein (or binder), the calibration material (or calibrator) and others such as separation material and buffer solutions.

[A kit may sometimes include key accessory items, e.g. membrane filters, which are necessary for the optimal function of the assay system.]

As the performance characteristics of a kit are governed by the interrelationship between the components and the (recommended) procedure used for carrying out the assay and as deviation from the recommended procedure may influence the performance characteristics and render the assay result invalid and unsuitable for its intended purpose. The kit shall be used according to the instructions included with the kit.

[Whilst the characteristics of suitable individual components can be specified to some extent, the ultimate suitability of each component can be assured only when it is tested with all the other components, and according to the full procedure which will be used for the kit in its final form.]

¹ The use in medicine of the Systè^me International d'Unités (SI) developed by the Conference générale des Poids et Mesures was endorsed by a resolution of the Thirtieth World Health Assembly in May 1977. A succinct, simple and authoritative account of the use of SI units entitled "The SI for the Health Professions" (World Health Organization, Geneva, 1977) is now available.

² Reference may be made to certain publications of the International Commission of Radiological Protection (ICRP). Particularly relevant are: Publication No. 5, Report of Committee V on the Handling and Disposal of Radioactive Materials in Hospitals and Medical Research Establishments, 1st edition, 1965, and the Safety Series published by the International Atomic Energy Agency, especially No. 1, Safe Handling of Radionuclides - 1973 edition.

1. Tracer labelled materials

All tracer labelled materials shall be prepared from the most suitable ligands available as determined by tests approved by the national control authority.

1.1 Tracer labelled ligand

The tracer labelled ligand shall be prepared from the ligand or an appropriate chemical derivative of the highest available purity.

√The use of pure materials reduces the assay non-specificity due to extraneous cross-reacting contaminants. Often the ligand requires chemical modification before or during the labelling process and the procedures used are determined by the type of label e.g. radio-nuclide, enzyme, fluorophor, or red blood cell label.√

The purity and suitability of the ligand shall be assessed both before and after labelling using a variety of physicochemical and immunochemical procedures approved by the national control authority.

√The tracer ligand is usually required to have a high degree of purity in order to produce an assay system of the required characteristics. There are some instances, however, where satisfactory assay systems can be developed with tracer ligands of moderate purity.√

The performance of the tracer ligand in the final kit is the most important factor. The tracer ligand selected shall possess sufficient stability to maintain an appropriate quality of performance over an acceptable and specified period of time.

1.2 Tracer labelled binding protein (tracer binder)

The tracer labelled binding protein used shall be approved by the national control authority.

√To date in almost every case where a tracer labelled binding protein is used it is derived from an antibody preparation, e.g. a purified immunoglobulin fraction, or antibodies obtained by affinity chromatography. In future antibodies from monoclonal hybridomas may become available. The selection of a preparation often depends on the titre of the original antiserum; a high titre and a low degree of cross-reaction with potentially interfering substances indicate potential suitability.√

The degree of purity of the final labelled antibody may vary considerably depending on the analyte being assayed, the required sensitivity and the design of the assay system.

Certain kits require a labelled antibody preparation which has been purified by affinity absorption such that more than 50% of it will bind specifically to excess solid phase antigen. Because of the nature of the procedure (number of wash steps, extremely low non-specific binding, etc) other kits may function satisfactorily with labelled antibody of a relatively low specific purity, attention having only been given to the removal of free uncoupled label.]

2. Antisera and other binding substances

The immunogens used for the production of specific antibodies shall be the purest preparations available. The method of production shall be approved by the national control authority.

2.1 Characteristics of the antisera

The antiserum used in a kit shall be characterized by tests suitable to uniquely identify it in order that there will not be batch by batch differences in performance.

[The characteristics required for an antiserum or other binding protein used in a kit will depend upon a variety of its properties, other constituents of the particular assay system in which it is used, the separation procedure and the physicochemical conditions in the assay system, such as the buffer and the type of test specimen.

In the case of substances of large molecular weight to be used as immunogens, the preparation should preferably be highly purified in order to avoid immunization with other closely related substances such as metabolic products of the analyte.

Alternatively, it may be possible to prepare monoclonal antibodies of high and constant specificity following exposure to an immunogen (even if impure) and an appropriate lymphocyte clone selected for the production of suitable antibodies.

In all cases, if an antiserum has been shown to be suitable for a given assay kit the nature of the immunogen used is irrelevant.]

2.2 Quality of the antisera

The quality of an antiserum shall be evaluated using the whole assay kit.

[An antiserum that is satisfactory when used with a certain tracer or separation system may not be suitable for use with another.

Adequate precautions should be taken to prevent gross microbial contamination of antisera and preparations of binding substances during manufacture; in many cases suitable preservatives (e.g. bacteriostatic or bacteriocidal agents) may be added to prevent product deterioration during storage, after re-constitution and during subsequent use.]

3. Reagents for separation of bound fraction and free fraction

Various techniques are used for the separation of bound and free antigens. The separation system chosen shall have been shown to work effectively with the kit when it is carried out according to the manufacturers recommended instructions.

[It is desirable for the separation system to be minimally influenced by such variables as temperature, time and pH, or by the nature of the biological fluid in which they exist. Furthermore, it should be relatively simple to operate.]

4. Reference sera

The performance of the kit shall be checked batch by batch by the use of reference sera.

[In the control of manufacture of kits it has been found of great benefit for the manufacturer to maintain a collection of reference sera with which to check the kit performance characteristics. Such sera normally consist of samples containing various concentrations, e.g. high, medium and low, of an endogenous analyte; sera to which measured quantities of analyte or calibrator have been added; sera containing no analyte (if possible without artificial removal of the analyte); and one or more examples of serum from patients with recognized pathology that the assay system might be used in association with (e.g. serum from patients with acromegaly for kits intended for assay of growth hormone). Such sera are useful for the qualitative assessment of specificity and parallelism; but they may also be used for checking the calibration of kits and for this purpose they may be assigned consensus values of analyte concentration, derived from repeated independent assays against the calibrator.]

Such reference sera shall be stored under conditions which will ensure maximal stability, e.g. aliquots stored in suitable sealed containers at or below -0°C or freeze dried.

National authorities shall provide or approve such reference sera for the calibration of the sera supplied by the kit manufacturers and recommend appropriate conditions of storage.

[Analyte values (if assigned) should be provided.

For similar purposes it may be desirable to provide for certain analytes one or more

reference sera for national or international use. For ease of distribution of such sera it is expedient to freeze-dry them in ampoules which can then be sealed, but it is essential to ascertain that the analyte has not been irreversibly altered by such desiccation. To obtain consensus values for such sera it is advisable to include samples of them in collaborative studies such as are used in setting up international or national standards (e.g. the preparation of postmenopausal plasma, NIBSC No.69/178, included in the international collaborative study of the International Reference Preparation of human pituitary FSH and LH, for bioassay).

Such reference sera should not be confused with quality control sera which are samples of sera included routinely in all assay series for purposes of within-laboratory quality control (internal quality control), or those distributed on a regional, national or international basis for between-laboratory quality control (external quality control).]

5. Reference materials

Reference materials used in the calibration of the kit shall include international and national reference preparations, the manufacturer's house standard and the calibrator(s) in each kit.

[The calibrator in the kit should contain the substance in the same molecular form as the analyte in the test specimen, or similar to it to the extent that it behaves in the kit assay system in the same way over the dilution range used, i.e. it shows parallelism (using a suitable statistical transformation) over the working range of analyte concentration for which the kit is intended to be used.]

Each sample of a specific lot of calibrator with an assigned value in the form supplied in each kit shall be identical.

[The stability of the calibrator should be estimated by accelerated degradation studies or other acceptable methods and shall be shown to be adequate for the temperature conditions likely to be encountered by the kit.]

The calibrator in the kit shall be calibrated in its final form by comparisons with the primary (e.g. international) reference material where appropriate.

[For this purpose, it may be necessary to use dilutions of such material in suitable analyte-free biological fluids (e.g. serum) which the kit is intended to assay. Calibration of house standard and of the kit calibrator should be based on the results of several independent

assays (e.g. starting with fresh dilution procedures) so that the value assigned to it is precise.]

In the case of peptides, proteins and other substances which cannot be completely characterized by chemical and physical means alone, WHO International Biological Standards and Reference Preparations shall be used for calibration; furthermore, these and International Reference Reagents, are used also for the assessment of the specificity ("analyte validity") of the kit assay system.

[Detailed description of the selection, preparation, ampouling, characterization and calibration of international and national reference materials is given in Annex 4 of the 29th Report of the ECBS.¹

Successive reports of the ECBS of WHO (published approximately annually) contain up-to-date information on the establishment of international biological reference materials. Technical information concerning the nature, characterization and calibration of each international reference material is normally sent with each distribution of ampoules, and additional information may be obtained from the International Laboratory responsible for its distribution.]

B. REQUIREMENTS FOR LABELLING KITS, COMPONENTS AND PACKAGE INSERTS

All components of a kit and the container of the kit shall be clearly identified by labels. The information given on the label on the container or the label on the package shall be approved by the national control authority. The labels on the containers shall be attached in such a manner that the contents can be seen and that the label is not easily detached. Any abbreviations used shall be approved by the national control authority.

1. Labels on the containers

The labels on the containers of the components, including reference materials, shall show at least:

- the name of the product.

[The name should be the non-proprietary name but a proprietary name may also be included.]

- the name of the manufacturer.

[This should be the registered name of the manufacturer or licence holder. Sufficient information should be included to allow a user of the product to make direct inquiries.]

- the lot number.

[This is a number or code from which the manufacturer can uniquely identify the manufacturing history of the components.]

¹ WHO Technical Report Series, No. 626, 1978, p. 101.

- storage and expiry date.

√The expiry date should be based on data obtained under specified storage conditions. Such conditions should form part of the statement of expiry date. Should the expiry date and storage conditions be different after reconstitution these should be stated.√

- a statement whether the component may or may not be used with other lots of the same kit or other kits.
- a caution to any hazards, if applicable.

√If any component may be confused with a product administered to humans a cautionary statement such as "for laboratory use only" must be used. If a hazard is associated with the product, cautionary words such as CAUTION or RADIOACTIVE should be used together with any internationally adopted symbol.√

In addition the label of a component that is an antiserum, shall show the animal species in which it was raised or whether it was produced in vitro in normal, transformed or cancer cell lines.

2. Labels on package

The labels on the package, shall in addition to the information shown on the label on the containers, show at least:

- the purpose for which the assay is intended.

√For example "Kit for estimation of free thyroxine in blood serum/plasma". Ambiguity should be avoided when a kit or material may have several uses, each requiring different concentrations or preparations, e.g. kit for estimation of human chorionic gonadotrophin in blood serum/plasma or urine; kit for estimation of free or total thyroxine in blood serum/plasma; kit for estimation of human placental lactogen in blood serum/plasma in the first, second or third trimester of pregnancy.√

- list of components and the number of vials, ampoules or bottles of each container in the kit.

3. Information given on the package insert or brochure

All kits shall contain a package insert or brochure that fully describes the kit including calibrators and gives precise instructions on the use of the kit. The information shall repeat the information given on the label on the package and in addition shall describe at least:

- the principle of the test.

√A concise explanation of the type (e.g. radio-immunoassay) and principle of the test; appropriate literature references should be given.√

- precautions.

[A description of known hazards and necessary precautions must be given. It is possible to give much more detail of the package insert than on the label on the package.]

- suitability for use.

[If appropriate and if the information is available, the physical, biological or chemical indications of instability or deterioration should be given. Where applicable, descriptions of appropriate tests that the user may apply to assure satisfactory performance should be included.]

- reagents.

The name and contents of reagents in the kit shall be stated when necessary for the proper performance of the procedure, the quantity, proportion or concentration of each ingredient, the generic source or sources, potency, specificity, avidity, sensitivity or other specifications as appropriate.

[For a product derived from biological material, the generic source or, if relevant, microbial group, type and strain should be given.]

A statement that the calibrator or kit has been calibrated against a specified recognized reference preparation and the estimated value stated.

- other materials.

The nature and characteristics of additional materials shall be stated.

[The characteristics of material such as a buffer, preservative or stabilizer could affect the proper performance of the test or influence the results.]

- list of materials supplied by the manufacturer.

[Special mention should be made of any components interchangeable with other kits and if so under what circumstances.]

- list of materials required but not supplied by manufacturer.

[Those materials not supplied by the manufacturer but required for the performance of the test must be listed. A description of the purity (e.g. analytical grade), procedures for diluting or mixing, and other pertinent information should be given to assess proper performance.]

- equipment.

[The specifications of any special equipment required should be given (e.g. type of rotor or gravitational force (g) needed for centrifugation).]

- specimens.

[Where applicable a description should be given of:

- (i) special precautions regarding specimen collection, including special preparation of the patient (e.g. for estimates of renin, the time of day and the position of patient)
- (ii) additives (e.g. preservatives necessary to maintain the integrity of the specimen)
- (iii) known interfering substances (e.g. a particular anticoagulant)
- (iv) recommended storage, and handling or shipping instructions for the protection and maintenance of the stability of the specimen.]

- instructions for use.

[Every detail of the operations required to accomplish correct results must be stated clearly and arranged in sequence of the operations. It is helpful to have such working instructions presented in a durable form suitable for continued use on the laboratory bench. Guidance should be given on:

- (i) precise experimental conditions that must be met e.g. pH, temperature, incubation times for specific steps of separation techniques
- (ii) calculation of results including an explanation of each factor and step in the calculation. If appropriate, an illustrative sample calculation should be given, including a typical dose response curve
- (iii) procedural techniques that may assist the user to perform the test more effectively (e.g. checking purity of water, pipette calibration, pouring supernate from precipitate compared with aspiration, proper sequence of addition of reagents, etc).]

- calibration procedures.

[Calibration procedures including the preparation of the calibration material as well as the construction of a dose response curve with the calibration material must be described.]

- precautions, during the conduct of the test.

√Any precautions that should be taken during the test in addition to those applicable to the components should be stated. These cover radiation, contamination with microbiological materials, disposal of waste material or chemical toxicity, etc.√

- validity.

√The following information should be included:

- (i) the advantages, limitations, precision, bias, etc. of the method
- (ii) for clinical application, expected results and details of how the data were derived, identifying the populations used, both for normal and pathological states
- (iii) a full description of usage of the reference materials and calibrators
- (iv) results should be expressed in units in common usage, where applicable SI units must be used. The confidence limits pertaining to any reported results and the method of calculation should be stated.√

C. GUIDELINES FOR ASSESSMENT OF THE PERFORMANCE OF IMMUNOASSAY KITS FOR HEALTH CARE

1. General

These guidelines are intended to cover the information which manufacturers shall have available for the national control authority. They are not entirely appropriate for the following situations:

- a manufacturer who may use the kit for additional measures for the assessment of the kit other than those that are specified in the Requirements.
- the practical assessment of kits by national control laboratories.
- the assessment of kits by individual users. This important topic is the subject of another WHO document in preparation.

Whilst the quality of the individual components of a kit is clearly important and will govern decisions during the assembly and manufacture of kits, the performance of the whole kit shall be the ultimate criterion for the quality of the kit and undue attention to the quality of the separate reagents may be irrelevant and counter-productive.

In all instances involving the processing of data from dose response curves or the statistical assessment of errors, details of the calculation procedures shall be made available by the manufacturer.

In principle the assessment of the performance of immunoassay kits does not differ from that of many other diagnostic kits in clinical chemistry, e.g. for urea. In practice, particular difficulties arise in the establishment of analytical validity (specificity and bias) and in the maintenance of assay to assay reproducibility.

It is recognized that a given manufacturer may produce various types of kits, each for a different analyte, or which sometimes may be applied to the measurement of more than one analyte.

- when a manufacturer supplies a kit the results from which may be used to assist clinical diagnosis, the intended clinical application must be stated, and the manufacturer is required to obtain evidence to support the statement.
- if, for a given clinical condition, no data are presented, it is implied that the manufacturer (at that time) does not offer the kit as suitable for that purpose, or has not completed validation for it. In such a case, should a user choose to use the kit for that purpose, he would do so on his own responsibility.
- a manufacturer may also produce a kit for an assay (of a stated analyte) for which there is no claim for its clinical usefulness, for example for the assay of a newly discovered compound whose clinical application is as yet unassessed. In this case it is the concern of the control authority that while the manufacturer may not make, or be able to substantiate any claim of clinical usefulness, such kits should be available for investigatory use providing that all the performance characteristics are fulfilled as for in vitro diagnostic kits, except for the justification of its clinical application.

The production of reliable kits for a new analyte can be of great importance in the evaluation of potential diagnostic application(s).

2. Assessment of random errors (precision) of immunoassay kits

2.1 Within-assay series precision and sensitivity

The reproducibility (precision) of replicate measurements in a given dose range in an assay will determine the reliable working range of that system. In immunoassays some of the errors leading to poor precision are a feature of the assay design, but others (e.g. poor pipetting, counting errors) are largely beyond the manufacturer's control. An important factor in kit design is that it gives maximal precision at analyte concentrations of maximal clinical interest, although it is appreciated that with the use of certain provocative tests e.g. the measurement of insulin following a glucose load, it may be necessary to recommend appropriate dilutions of the test sample.

The precision of repeat estimates within one assay series of specimens covering the clinically relevant analyte concentrations of the dose-response curve shall be given. This "precision profile" indicates the working range of the assay. The detection limit (sensitivity) of the assay is the least amount that can be differentiated from zero analyte with a designated probability. Particular attention should be given to the ranges of analyte concentration considered normal and those at which clinical decisions will be made. At this stage, data obtained by the manufacturers are used, since data from user laboratories could be misleading. Details regarding the number of replicate determinations and the number of occasions upon which the precision was measured (perhaps including different batches or filling lots of critical reagents) shall be given.

2.2 Between-assay series precision (reproducibility)

Between-assay series precision can be affected by many factors, such as change of operator, change of component or even a different filling lot of a component. The extent to which an assay is insensitive to such factors is called ruggedness.

The manufacturer shall provide details of reproducibility and inform the control authority of the method used to assess it. As a guideline, between-assay variation shall be assessed at a minimum of 3 dose levels covering the range of analyte concentrations likely to be encountered in clinical applications (preferably including those at which clinical decisions may be made). Between-assay variation shall be assessed for the shelf-life of lots and batches of critical reagents, and from lot to lot and from batch to batch of the complete kit.

3. Assessment of systematic error (bias) of immunoassay kits

Evaluation of the validity of immunoassay kits involves important matters of principle and practice. For many analytes (e.g. peptide hormones) there may be no ascertainable "true value" in strict analytical terms against which the bias of a kit can be assessed. Even if there is such a value, it may not be clear how it should be assigned. For some analytes with a well-defined chemical structure, so-called reference methods may be available, e.g. mass spectrometry, or isotope dilution mass fragmentography for steroids and other small molecular weight compounds. But "reference methods" should be used with care by control authorities as at present there are few data about the precision, reproducibility and bias of the methods in use.

Many other procedures are used to evaluate the specificity of a kit assay system and these shall be agreed with the control authority. The following are useful criteria but each in itself is insufficient to demonstrate validity.

Parallelism of dilutions of analyte and calibrator

Recovery of known amounts of added reference material

Comparison with other methods, e.g. after further purification by chromatography

Consensus with values obtained by other methods or other kits, for specimens obtained from well-defined physiological or pathological situations.

4. Specificity

Specificity of the binding protein indicates the potential specificity of the complete kit but is, in itself, insufficient to ensure that the kit has suitable specificity. In certain circumstances the effect of certain factors (e.g. the nature of the specimen of biological fluid, the effect of interfering drugs and metabolites) may need to be determined. It is clear that all possible factors that could influence kit usage cannot be investigated initially, but information comes with experience of use of the kit. Manufacturers should collect evidence on possible interference by well-known drugs and especially by those substances that are chemically related to the analyte, including possible metabolites of the analyte. The attention of kit users should be drawn to the publication¹ of the American Association of Clinical Chemists on the effects of drugs on analytical assays.

5. Clinical effectiveness of a kit

If a diagnostic kit is developed and supplied for a specified clinical objective, then the effectiveness of the kit in achieving this objective shall be part of the assessment. Clearly this effectiveness will depend in large measure on the qualities indicated under headings 2 to 4 above.

¹

Effects of Drugs on Clinical Laboratory Tests, Clin. Chem. (1975) 21, No. 5.

In certain cases the effectiveness of a kit which gives a Yes/No result (e.g. test for pregnancy or for hepatitis antigen) can be assessed by a relatively simple clinical trial and the data presented in the form of rates of false negative and false positive results.

In the case of kits intended for a stated clinical purpose, the expected analyte concentration ranges in the normal population (normal range) and in defined clinical conditions should, where feasible, be given. It is important for control authorities in different countries to appreciate the extent that analyte concentration ranges in normal populations may differ from country to country (because of genetic, dietary, environmental and other differences) and within population groups within a country (because of age, sex, occupation, and other differences). It is beholden upon the user to confirm or establish normal ranges for the specific population in which the test is being used.

The clinical effectiveness of a kit may also depend on other factors unrelated to the efficiency of the kit per se. The manufacturer should assess and provide information on the effect of these factors, which include:

- possible differences between estimates in plasma and serum, and the effect of anticoagulants.
- conditions under which the specimen should be collected (e.g. fasting, time of day, posture), separated (e.g. rapidly, cold), and stored (e.g. frozen at -20°C or below).
- effects of interfering drugs and other substances (e.g. binding globulins in pregnancy) either on the assay system itself or on the metabolism of the analyte.

6. Clinical testing of assay systems

6.1 Assay systems previously well described

The clinical testing of assay systems previously well described and of endogenous substances for which information on their physiology is available: The expected range of normal values in a specified suitable population shall be stated including ranges obtained for stimulation (provocation) and suppression tests if this is the proposed use of the kit. Such analyte ranges should be specimens taken from adequate samples of well-specified populations and apparently normal subjects, under carefully defined conditions. In addition, reference to, or discussion of, the influence of other stimuli that may be relevant should be cited.

Pathological responses or values: for disease states the test kit is designed to study, adequate sampling is required under defined conditions in a specified and preferably matched population, to permit reliable differentiation from normal. The distribution of values obtained in normal populations and the expected values in diseased populations should be stated. Any overlap in the ranges in such populations should be stated.

6.2 Assay systems for analytes previously undocumented

The clinical testing of assay systems for analytes whose role in health and disease has not previously been documented: Results of assays carried out on samples of apparently normal subjects should be provided as evidence to support claims that this procedure might have a therapeutic or diagnostic application. Even when such applications are not known or claimed, evidence for the specificity of the assay system for the analyte must be supplied. Any known limitation of the assay system or its application must be stated. When a clinical application is claimed the recommendations in section 3.4 must be followed.

7. Clinical assessment of kits for the assay of drugs

In the assessment of an assay system for its use in assays of drugs, the drugs administered shall be of a quality approved by official agencies such as established pharmacopoeias. International or national or, if these are not available, other well-recognized reference materials, shall be used in the assessment of assays of drugs. The drug should be administered in doses that provide adequate but not excessive therapeutic concentrations of the drug in plasma. The ability of the kit to assay the drug at plasma concentrations at which clinical decisions would be taken must be shown by adequate sampling under well-defined conditions in specified populations. Knowledge of the pharmacodynamics and metabolism of the drug is helpful and desirable. Information provided should include statements of the doses and dose forms of the drug, time and route of its administration, and the times when test specimens for assay were obtained.

8. Stability of kit components and kit performance

The manufacturer shall assess, e.g. by accelerated degradation studies or other appropriate methods on the freeze dried material and on the reconstituted solutions under storage conditions, the stability of (1) the individual components of the kit, before and after reconstitution (in the solution as directed), and (2) the assay performance characteristics of the whole kit when used according to the manufacturer's instructions. Information about such testing, the number and results of the tests and the statistical procedures used for their evaluation shall be made available to control authorities.

The performance characteristics for the shelf-life of the kit described above shall be provided. Kit batches incorporating different filling lots of components (e.g. analyte tracer) shall also be tested and the number of such tests, their results and the statistical procedures used shall be stated.

Assay to assay reproducibility will be affected by the stability of the kit; in part this will be a result of the changing bias or specificity of the kit as the least stable component deteriorates.

GLOSSARY OF TERMS USED IN IMMUNOASSAY METHODOLOGY

ACCELERATED DEGRADATION STUDIES

Studies in which an estimation of rate of change of for example a reagent is made by comparison of samples of the reagent which have been subjected to conditions (e.g. high temperature) that increase the rate of change, with samples of it held under conditions at which such changes are minimal, e.g. low temperature. See STABILITY, page 26.

ANALYTE

Substance in test specimen to be quantified.

ANALYTE TRACER

Analyte labelled with tracer.

ANALYTE VALIDITY

The specificity of an assay system for estimation of a given analyte in a given type of test specimen, e.g. a specified biological fluid.

ASSAY KIT

A set of components (reagents and other necessary materials) and procedural instructions packaged together and designed for the estimation in vitro of a specified analyte to a stated degree of precision when used according to the recommended instructions.

ASSAY PERFORMANCE REQUIREMENTS

Assay performance characteristics required for a particular (specified) purpose.

ASSAY SERIES

A set of estimates determined with the same reagents and reagent solutions under the same uniform conditions; thus a group of assay tubes (of calibrator, test, blank and control samples), which are handled together at the same time under the same conditions.

ASSAY SYSTEM

All the components and procedures of an assay.

ASSAY STANDARDIZATION

Measures required to ensure uniformity of assay results. Standardization enables improved reproducibility of assay results and valid comparability by reduction of bias between assay series or between laboratories. One measure usually taken (and often essential) to achieve this is the use of a calibration material (standard); in protein binding assays (and many other assays involving biological systems) this may be insufficient by itself to ensure uniformity. In such instances it may be necessary to define the assay reagents and procedure.

BATCH

A collective noun for a group of specimens or containers processed together in a uniform way. The material in the containers may be similar, as in a uniform filling lot (as in pharmaceutical practice) or different, as in an assay series of various specimens.

BETWEEN-ASSAY VARIATION (REPRODUCIBILITY)

Extent to which a set of replicate measurements obtained with an assay series agrees with measurements from previous assay series (obtained with the same assay system). Expressed quantitatively as standard deviation or coefficient of variation and may be referred to as between-assay series variation. Reproducibility may be dependent on dose level and thus may vary at different analyte concentrations.

BIAS

The numerical difference between the average of a series of estimates and the true or accepted value.

BINDING PROTEIN, BINDING REAGENT, BINDER

Protein used to quantify or detect an analyte by virtue of its ability to bind with it. Protein that reversibly and non-covalently binds a ligand and may be used to quantify or detect an analyte by virtue of its ability to bind with it. Examples include antibody, hormone cell receptor protein, and plasma proteins that bind small molecules.

BIOLOGICAL REFERENCE MATERIAL (or "biological standard")

Material containing a substance generally of biological origin which cannot be, or is not, characterized completely by chemical and physical means alone.

The term "standard" is also often used to mean a set of specifications of the quality of reagents or assay kit, or quality of assay performance.

BIOLOGICAL STANDARD

See above.

BOUND TRACER

The fraction of the total tracer present in the protein bound fraction after separation. Normally includes non-specific binding and background signal. See also BOUND FRACTION, below.

BOUND FRACTION

The fraction of all the reactants present in the protein bound phase after separation.

CALIBRATION CURVE, CALIBRATION DOSE RESPONSE CURVE

The term "standard curve" has been used, but should be discouraged wherever there could be ambiguity.

The graphical relationship between the amount (dose metameter) of reference material (horizontal axis) and the response of the detector (vertical axis).

The dose response curve (qv) of the calibration material in an assay series.

The dose may be expressed in terms of amount of specified substance (mole) or mass of the reference chemical, or in terms of amount ("units") defined by a specified material.

- dose metameter is a numerical transformation of the dose variable, for example log dose.

The response variable can be, for example, the (uncorrected) activity signal in the free or bound fraction.

CALIBRATION OF KITS

Steps taken (by manufacturer or laboratory) to ensure that the results of assays obtained with an assay kit are expressed in terms of mass of a pure chemical or for substances which are incompletely defined, in terms of units defined by a reference material (qv).

CALIBRATION MATERIAL, CALIBRATOR, OR CALIBRANT

Component of a kit with which the test specimen is compared in order to determine the concentration of analyte.

CARRIER

1. Substance added to stabilize assay reagents, e.g. by prevention of non-specific absorption to surfaces, but which is otherwise inert toward the other reagents and assay system. Analyte-free serum or plasma or enzyme-free albumin is generally used.
2. Also used for particle, solid phase of macromolecule.
3. In radiochemistry, non-radioactive nuclide present or added to dilute radionuclide, e.g. to minimize absorption or biological uptake.

CLINICAL EFFECTIVENESS

Usefulness of the result of an assay for the clinical purpose for which it was carried out.

CONSENSUS VALUE

Value (e.g. for concentration or activity) derived from many, preferably independent, assays or observations.

CROSS-REACTION

Ability of substances other than the analyte to bind to the binding reagent, and ability of substances other than the binding reagent to bind to the analyte.

Such substances if present in a test sample may compete with the analyte for the binding site - and thus lead to an erroneous potency estimate.

These substances may be natural precursor forms of the analyte or binding protein, degradation products (from in vivo or in vitro degradation) or other substances which carry on their surface a molecular configuration similar to the binding (immunoreactive) site(s) of the analyte or binding protein.

DETECTABILITY, DETECTION LIMIT

The smallest amount or concentration of analyte which with a stated confidence (commonly two standard deviations, or expressed as confidence or fiducial limits) can be distinguished from zero. This value depends upon the precision of the measurements of zero dose solution and of the specimen (See SENSITIVITY, page 25).

In some detector systems the detection limit is determined by the signal-to-noise ratio in the measurement device.

DOSE RESPONSE CURVE

The graphical relationship between the amount of reference material or specimen (horizontal axis) and the response of the detector (vertical axis). See also CALIBRATION CURVE, page 19.

FILLING LOT

Group of containers filled (with a reagent) under uniform conditions.

FREE FRACTION

Those components in the incubation mixture which are not present in the bound fraction. See BOUND FRACTION, page 19.

HOUSE STANDARD

Reference material (qv) generally prepared and used by a single manufacturer or laboratory.

IMMUNOASSAY

Term used for an assay procedure based on the reversible and non-covalent binding of an antigen by antibody. Immunoassays can be employed to detect or quantify either antigens or antibodies.

IMMUNOGEN

Substance which when administered under appropriate conditions to a suitable animal will stimulate the production of antibody, or antibodies that can combine reversibly and non-covalently with the immunogen as antigen.

All immunogens are antigens; however, not all haptens are immunogenic and able to stimulate a specific antibody response, cf. haptens.

IMMUNOREACTIVITY

A term commonly used for the ability of a specified antigen to combine with an antibody, or a specified antibody to combine with an antigen.

The term immunological activity is loosely used for the concept of (1) the ability of an immunogen to elicit an immune response (e.g. to raise antibodies); (2) the "potency" of an antigen exhibited in an immunoassay; or (3) the potency of an antibody in an immunological reaction.

INDEPENDENT ASSAY

Assay in which fresh solutions and dilutions of assay reagents are used (contrast with replicate assay).

INTERFERENCE

Effect of factors or substances other than the cross-reactants which bias assay results either by affecting the kinetics of the reaction or by altering the efficiency of the separation procedure.

The term "non-specific interference" has also been used.

Requires to be distinguished from "cross-reaction" (qv) which refers only to substances (other than the analyte, labelled antigen and antibody) interfering with the binding reaction due to steric similarity.

INTERNATIONAL REFERENCE MATERIAL

Reference material (qv) distributed on an international basis by a recognized international, (e.g. WHO), or sometimes national organization.

KIT - See ASSAY KIT, page 18.

KIT COMPONENT

Reagent used in a kit. Primary components of a typical kit are (1) the binding reagent (qv); (2) the tracer (qv); and (3) the calibrator; secondary components include the separation materials, buffers and quality control sera if included.

LABEL

1. Paper bearing information attached to the immediate container of a reagent.
2. The substance (e.g. radionuclide, fluophor or other) attached to one of the assay reactants for the purpose of facilitating observation of the binding reaction by yielding a perceptible signal.

LABELLED BINDING PROTEIN

Tracer, qv, in which the labelled reactant is the binding protein.

LABELLED LIGAND

Tracer in which the labelled reactant is the ligand.

LIGAND

Substance that is reversibly and non-covalently bound by a binding agent. A general term used for analyte, cross-reactant or calibrant which binds to the binding reagents.

MASS CONCENTRATION

Concentration of a substance expressed in mass/unit volume, e.g. g/l, (as SI practice).

MAXIMUM BINDING

Term used in 2 senses:

1. The maximum amount (usually expressed as a percentage) of labelled ligand bound by the binding reagent at its working dilution in the absence of added calibrant or analyte (zero dose binding, qv).
2. The amount (usually expressed as a percentage) of labelled ligand bound to excess binding reagent.

MISCLASSIFICATION

Extent to which free (i.e. non-bound) material appears in the bound fraction, and vice versa.

MISCLASSIFICATION ERROR (see NON-SPECIFIC BINDING, below)

Variability of misclassification. Non-specific binding represents one form of misclassification.

MONOCLONAL ANTIBODIES

Antibodies derived from a single clone of lymphocytes.

NON-SPECIFIC BINDING

The fraction of labelled material present in the bound fraction for reasons other than specific binding to the binding site of a binding protein. See also MISCLASSIFICATION, above.

NORMAL RANGE (of analyte concentration)

A range of concentrations which includes a stated percentage (usually 95% or 99%) of a defined population group (qv). The normal range may be one-sided (e.g. when only high values are considered abnormal), or two-sided (excluding, say, the lowest $2\frac{1}{2}$ and the highest $2\frac{1}{2}$ of the population) when both high and low concentrations are of clinical interest.

The normal range is most satisfactorily estimated from the percentiles of the set of concentrations obtained for a group of individuals, chosen to represent the defined population. This takes into account any skewness of the distribution of values. The precision with which the normal range is estimated will depend on the number of individuals in the group - and confidence limits can be assigned to the boundaries of the normal range. The normal range may be biased if the group of individuals is not a random sample of the defined population - i.e. all members of the population do not have an equal chance of being included in the population.

OPERATOR

Person carrying out the assay.

PARALLELISM

Extent to which regressions of response (or of response metameters) on dose (or on dose metameters) of two substances are identical except for (horizontal) displacement of one relative to the other. If the regressions are straight lines, the extent to which they are parallel. If the regressions are curvilinear, this condition is described as generalized parallelism. Parallelism is one test of identity of analyte with calibrant. Traditionally it was a prerequisite for the calculation of a single valid value of relative potency of one substance compared with another in an assay.

PERFORMANCE CHARACTERISTICS

Properties of an assay system relating to reliability and practicability. Characteristics of the reliability of an assay include precision, bias, sensitivity, specificity, validity and ruggedness.

Characteristics of the practicability of an assay include speed, technical simplicity, cost, resources required, service availability.

POPULATION GROUP

A population defined with regard to relevant factors such as age, sex, geography, ethnic group, drug treatment, social levels and the physiological status of the subjects.

POPULATION RANGE (of analyte concentration)

The range of estimated concentrations of an analyte (or presumed analyte) in a stated biological fluid, assayed in a particular manner, which is found in a specified population. The population (qv) may be defined with regard to relevant factors such as age, sex, geography, ethnic group, diet, drug treatment, social levels, the physiological status of the subjects and the time, manner and conditions in which the specimen of biological fluid is withdrawn.

PRECISION/IMPRECISION (of a measurement)

Imprecision represents the measurement of disagreement between a variability of replicate measurements (of the same material). It is usually represented by the standard deviation or variance or coefficient of variation. Such variability can occur within a single analytical series, between assay series, between batches of reagents, between operators or laboratories, and between assay systems. Confidence limits or fiducial limits quantify the uncertainty about the value of a parameter after estimation from an experiment or sample.

Strictly speaking, precision is the inverse of imprecision and represents the degree of agreement of replicate measurements; but in practice it is widely used instead of imprecision to describe variability.

Whenever a figure for variability is given, it should be stated whether it applies to a single assay series, between-assay series, between batches of reagents, between operators or laboratories, or between assay systems.

In protein binding assays, the variability of the estimate(s) may depend on the analyte concentration(s) measured. (see PRECISION PROFILE qv, below)

PRECISION PROFILE

Graphical representation of precision (or imprecision) of measurements by an assay system over a range of dose levels of the analyte. Such profiles are of value in assessment of the reliance that can be placed on an assay estimate at a particular dose level, because the precision in binding assays may vary considerably with analyte concentration.

In assays where the precision does not vary over the dose range used (homoscedasticity), a single index of precision can be given.

QUALITY ASSURANCE

All documented test and evaluation procedures routinely performed on raw materials, in-process materials, completed components and the final test kit, together with all records and results which ensures that the product and the materials from which it is made, meets pre-determined validation, quality and acceptance criteria.

QUALITY CONTROL - INTERNAL OR WITHIN LABORATORY

Steps taken in a laboratory to ensure that the assay system is giving acceptable and reproducible values, normally involving the measurement of precision and bias. Such procedures are utilized within a specific assay series or between assay series.

QUALITY CONTROL - EXTERNAL OR BETWEEN LABORATORY

Quality control undertaken by an outside independent agency to assess the performance of an assay by a group of laboratories. It includes the monitoring of assay performance in individual laboratories and assessment of the comparability of results between laboratories.

QUALITY CONTROL SERA

Sera used to assess and control quality of (assay) reagents or assay performance. Includes serum samples included in assay runs (to assess between-assay and between-laboratory variability). Also used by assay kit manufacturers for the assessment of the quality of an assay reagent and assay system.

RECOVERY

The fraction (percentage) of analyte remaining in a sample following a procedure (generally) carried out to purify or concentrate the analyte.

Usually estimated by addition of known amount of radioactive or inactive analyte.

The term is also used for the amount estimated by an assay system expressed as a percentage of the total amount known to be in the sample; it is usually assessed by estimation of the amount of analyte in a sample after the addition of a known amount of (unlabelled) analyte. The discrepancy between this and 100% represents a measure of the recovery and thus the bias of that assay.

REFERENCE MATERIALS

Non-specific term for calibrator, "standard", reference preparation or reference reagent. Such reference materials may be distributed on a laboratory, regional, national or international basis.

REFERENCE PREPARATION

Identified preparation of a reference material of attested suitability containing a specified analyte and intended for assessment of quality or quantitation of an assay system.

Essential prerequisites for a reference preparation are that each sample of it is identical to another, and that its stability should be measured and suitable for its intended use.

REFERENCE SERUM

Serum containing one or more specified analytes, which are accepted as suitable for assays for the comparison and assessment of bias and reproducibility of different assay systems. Such a serum may be distributed on a laboratory, regional, national or international basis.

REPLICATE ASSAY

Assay in which the same set of solutions and dilutions of assay reagents are used; (contrast with independent assay).

REPRODUCIBILITY (of results of an assay system)

Extent to which a set of replicate measurements obtained with an assay system agrees with subsequent sets obtained with the same system.

Reproducibility can be quantified as the standard deviation or coefficient of variation and may be referred to as between assay series variation. Reproducibility should be measured at different dose levels.

ROBUSTNESS

Insensitivity of estimates and statements of precision to small variations in assumptions used in statistical analysis such as choice of metameters and form of dose-response relation.

RUGGEDNESS

Characteristic of an assay system which make the results obtained unaffected by changes in assay reagents and procedures.

Ruggedness can be assessed experimentally by observation of the influence on assay results (either from assay tube to assay tube or from assay batch to assay batch) of changes in the amount or quality of the assay reagents, or in the assay procedure.

In practice, non-ruggedness is manifested by poor precision, poor inter-assay variability and poor inter-laboratory agreement.

SENSITIVITY

Often used as synonym for detection limit (qv) or detectability.

SEPARATION MATERIAL

Reagents by which the labelled tracer fraction is separated from the bound fraction in a binding assay system.

SEPARATION PROCEDURE

Procedure by which the free fraction is separated from the bound fraction in a protein binding assay.

SEPARATION SYSTEM

Reagents and procedure used to separate bound and free fractions.

SHELF-LIFE (of batch of kit)

Period until recommended expiry date of the batch of kit.

SPECIFICITY, ANALYTE SPECIFICITY

Specificity (structural) of a protein binding reagent is the degree to which it is not influenced by cross-reacting substances.

Specificity of an assay system is the degree to which the results are not influenced by cross-reacting substances, or other substances such as plasma proteins and small ions present in the system, or other factors such as the pH or temperature of incubation.

STABILITY

Lack of alteration under defined conditions. Thermal stability of for example a reagent may be quantified as rate of chemical change at stated temperature(s). Often measured by accelerated thermal degradation studies, (qv) by comparisons of samples of a material maintained at different temperatures for one or more period(s) of time.

SUBSTANCE CONCENTRATION

Concentration of a substance of known chemical structure expressed as moles/l.

TEST SPECIMEN OR SPECIMEN

Specimen or sample (e.g. of biological fluid) in which it is required to detect or quantify the analyte. The terms "unknown sample" or "unknown" have frequently been used for this.

TITRE

Term for the volume fraction (or dilution) of a specimen or other solution at which a specified effect or end point is observed. Usually expressed numerically as the reciprocal of the dilution.

In the context of an immunoassay titre is usually used in the assessment of antiserum for the final dilution at which a specified substance fraction (percentage) or a specified amount of a specified analyte is bound.

The numerical value of a titre, of for example an antiserum, is thus influenced by the amount and affinity of antibodies and the conditions under which the test is made.

TRACER

The tracer is the reactant (ligand or binding protein) which incorporates a label which is used to trace the distribution of the unlabelled reactant in an assay system.

WITHIN-ASSAY SERIES PRECISION

Precision determined in an assay series.

WITHIN-ASSAY SERIES SENSITIVITY

The detection limit determined in an assay series.

WORKING RANGE OF ASSAY

Range of analyte concentration in specimens for which the assay system gives results with precision acceptable for the purpose (e.g. clinical) for which the assay was carried out.

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