International Federation of Clinical Chemistry (IFCC)

Fundamentals for External Quality Assessment (EQA)

Guidelines for Improving Analytical Quality by Establishing and Managing EQA schemes. Examples from basic chemistry using limited resources.

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1. INTRODUCTION

This document is written to assist colleagues in establishing and managing external quality assessment (EQA) schemes at an early stage. The document deals with frequently performed clinical chemistry analyses. However, many of the aspects described also apply to EQA in other branches of laboratory medicine. Special attention has been paid to the resources needed so that the document can be used in developing countries. We have also included basic educational material to enable scheme organizers to help participants interpret their results, and to assist participants to use EQA data to support and supplement internal quality control (IQC). The appendices of this document are arranged so that the early appendices are of interest for many of our colleagues while the later appendices are mainly of interest for colleagues specialising in EQA work.

Although the major topic of this document is EQA, we believe it is essential to encourage an integrated view of quality in clinical laboratories, so that any aspect of quality is seen as a part of total quality management. Thus IQC and EQA will be seen as components of an overall quality policy which includes other quality issues such as turnround times, gross errors, the training of laboratory staff, staff safety, and the appropriate use of the laboratory by clinicians including the interpretation of data.

The value of EQA in highlighting areas of analytical inadequacy and for stimulating improvements in between-laboratory variation has been demonstrated in many countries. EQA surveys in countries without EQA programmes or effective IQC have shown that up to 50 % of laboratory results for basic clinical chemistry tests are so far from the target value as to be of no clinical use. The cost of setting up an EQA programme must be viewed against the resources wasted in producing inaccurate data and the improvement in patient care which can be achieved by improving the quality of laboratory results. EQA must not be seen as either an alternative or a competitor to IQC: both are essential tools for a clinical laboratory. IQC is used in the decision to accept or reject results on patients' samples and enables the laboratory to describe and monitor the quality of its work; EQA permits a comparison of quality between laboratories and thus describes the "state of the art" for that area of laboratory work encompassed by the EQA programme. Rightly used and understood, EQA programmes will stimulate the development of quality assurance including IQC within participating laboratories.

Many EQA programmes are organized by the national professional scientific society or by professionals on behalf of government, although others are run by commercial companies. Whichever way it is organized several policy decisions need to be agreed professionally prior to the commencement of the programme. For example, the level of anonymity of the data must be agreed. With the use of computers it is possible to secure a very high level of confidentiality; however, this may entail an inappropriate level of investment for some countries and agreement may be reached so that the scheme organisers will have access to the performance of individual laboratories but maintain the confidentiality of the results.

EQA programmes require running costs, for example, for provision of the material, mailing expenses and clerical and professional time for administering the scheme. There needs to be agreement within the country or area about the finance; will it be funded by participants, by government, or in some other way.

Other issues also need agreement such as the frequency of specimen distribution and the extent to which there will be interaction between the organisers and participants to ensure an understanding of the scheme's objectives and reports.

We believe that EQA schemes have great value for the assessment of quality, and rightly constructed can provide data which can form a part of a national or regional laboratory certification, accreditation, or licensing procedure. However, the limitations of EQA must also be identified and understood. It should be stressed that there are many other potential sources of laboratory error apart from those which may be detected or controlled through an EQA scheme. It is vital that these are identified and that effective quality assurance procedures are in place to ensure that scarce laboratory resources of materials and personnel are used to the best advantage for the care of patients.

For EQA schemes to be successful in providing independent, objective data and to act as an educational stimulus to improvement, partipants must have confidence in the scientific validity of the scheme design as well as the reliability of its operations. The scientific validity will include stable, homogeneous specimens which behave like clinical specimens (proper commutability), and valid target values; reliability involves keeping to a strict time schedule with rapid feedback of initial performance information after analysis, structured and intelligible reports, and a cumulative data system.

2. SCHEME

2.1 Organizer

The selection or approval of an organizer and support facilities for an EQA scheme is an important process that will, inevitably, affect the quality, scope and subsequent success of the scheme. Ideally, this individual will have an established reputation in the field that the survey activities will cover. However, it is more important that the individual is committed to energetic long term involvement in the scheme, and demonstrates the qualities of vision and diplomacy that will be so important **during** the difficult periods that an emerging or developing scheme will experience.

If the EQA scheme is to be supported by government or institutional funds, it is imperative that the organizer has the "official" support of that agency. At the same time it is important that sponsoring agencies do not "over-influence" or inhibit the development of a scheme, or create an environment where potential participants are hesitant to be involved because of real, or apparent, disciplinary consequences.

It is also important that an organizer has the support of, and has his/her activities monitored by, an Advisory or Organizing Committee. With appropriate selection, the committee will serve to eliminate the perception that arbitrary criteria are used to assess performance, and will aid greatly in the important task of communication.

2.2 Scope

Determining the scope of an EQA scheme is crucial during the period when it is being established. Inevitably, the success of a scheme will provide opportunity to widen the scope, but it is important to note that most of the large schemes that operate successfully today in Western Europe and the United States started in a very simple form. Therefore it is essential to draw up realistic, and practical, limits to the field of analyte assessment (e.g. routine chemistry tests, haematology, toxicology or endocrinology). If no experience exists in the country concerned, or if the organizer, or committee have limited experience, it is far more practical to resolve the difficulties in mailing, packaging, report form

design, result recording and the production of helpful reports without the added complication of preparing complex materials for distribution.

The scope of the scheme also includes the number of potential participants, and their geographical location. In the early stages of a scheme it is important to achieve success, for example within the capital city, in conquering the logistic hurdles of specimen acquisition, specimen distribution and fast turn around of meaningful results. However, circulation in more rural areas with less easy communication should also be attempted to gain experience. Growth of the scheme to increase the number of participating laboratories or the number and scope of analytes can come later..

At the commencement of the scheme, a well formulated plan addressing the frequency of distribution must be determined by the organizer. The frequency of specimen distribution will affect the cost of the scheme, the enthusiasm of the participants and the administrative workload of the organizer's office. Several alternatives exist, and choices should be made that optimize the distribution frequency and turn- around time, see Table 1. Those intending to start a National External Quality Assessment Scheme (NEQAS) are recommended to inform the Unit of Health Laboratory Technology, World Health Organization, Geneva, Switzerland, see D5 in Appendix D.

Table 1. EQA scheme, examples of distribution frequencies and turn around times

Distribution frequency of specimens	Turn around	Benefits	Problems
1. Assayed immediately			
Quarterly*	< 14 days	Low administrative load Easier specimen acquisition	Less benefit to laboratory
	> 30 days		Turn around time is too long
Monthly	< 14 days > 30 days	Frequent information	Increased cost Turn around time is too long
Every 2 weeks	< 14 days	Very frequent information	High cost Administration Specimen acquisition
2. Delayed assays			
Annually, but requiring weekly or monthly assays	As soon as possible	Low distribution and packaging cost	Specimen storage Specimen acquisition

* minimum frequency recommended

2.3 Logistics

The most successful schemes are those in which participants can expect regular dispatch of speci-mens and fast turn around of results. Accordingly, operation of the organizer's office must be efficient to prepare, or acquire specimens, arrange their packaging and distribution and deal expeditiously with the documentation that is the basis of an EQA scheme.

2.3.1 Planning

Table 2 provides a list of questions that need to be considered before implementing an EQA scheme. The questions are designed to ensure that the creators of new schemes explore the full consequences of their choices and anticipate as many problems as possible.

Table 2. Check list for use before starting a EQA scheme.

XXXYYY External Quality Assessment Scheme	Check	
What field? (e.g. Chemistry/Toxicology/Endocrinology)		
How many laboratories?		
What geographical area?		
Is anonymity assured Who holds the key to the code?	Yes Yes	No No
What analytes, initially?	List	
What type of specimen? (serum/other; liquid/lyophilized)		
With what frequency will specimens be distributed?		/Year
What "turn around time" is required?		Days
Who will design the forms?		
Does the Organizer have access to computer and software to facilitate record keeping?	Yes	No

Where will the specimens come from?
    Will Organizer manufacture?
    Can they be donated by Industry?
    Will they be purchased?
    Is there a need for Import License?
	
	Yes
	Yes
	Yes
	Yes
		
		No
		No
		No
		No

What containers will be used for specimen distribution?		
What are Postal Regulations relating to shipping of biological specimens?	Check	
Will forms be pre-paid for return of results?	Yes	No
How will results be recorded and results calculated?		
What criteria will be used to determine "range of acceptable results"?		
What will be the format of the report sent to participants?		
Is participation in the scheme mandatory according to regulation?	Yes	No

What is the budget for the EQA Scheme?
 	
    Do sponsors specify conditions?
    For what period is the scheme guaranteed support?
 	
    Are participants required to pay?
 	
    How much?
	
	Yes
	
	Yes
		
		No
		Years
		
		No

2.3.2 Specimens

It is recommended that early distributions of specimens employ commercial quality control products if these can be acquired, see section 3.2. There are several methods of preparing serum/plasma for distribution. Attention is drawn to the method described in Appendix F for the low cost production of ethanediol stabilized serum, particularly for organizers with limited budgets or those subject to import restrictions. Such materials are also suitable for IQC of many procedures. This topic is addressed more fully in Section 3, which also gives advice on the acquisition of commercial quality control products from industry.

2.3.3 Instructions

The provision of well written information and instructions is fundamental to the efficient operation of an EQA scheme and adequate advanced information should be given to all potential participants, possibly through a workshop before the scheme starts. Experience has shown that participants will frequently carry out incorrect assays, or fail to meet time limitations unless good communication is achieved. The information provided to potential participants must describe in full the aims and objectives of the scheme, and ensure that all understand the following:

- the scope of the scheme (i.e. number and geographical distribution of participants);
- the need for regular participation;
- the method of result assessment;
- procedures and criteria to be used for peer comparison;
- sources of advice for laboratories experiencing problems with assays.

The instructions given to participants regarding the assays should ideally accompany each specimen that is distributed to ensure that conditions on storage, handling, timing, and assay are all met. An example of sample handling is presented in Appendix A.

Liaison with participants is important for a successful scheme and should be promoted through regular (e.g. annual) reports and requests for feedback, and, if possible an annual meeting on quality assurance bringing together the organizers and participants. Participants should view the scheme as theirs, rather than one imposed by a distant bureaucracy.

2.3.4 Distribution

The frequency of distribution will be dictated largely by economics; however, the following need to be considered when assessing the distribution operation:

- postal regulations governing-shipment of biological materials as well as mass and outer dimension of the package;
- type, number and volume of specimens to be distributed;
- package that can accomodate all specimens and forms needed
- package that facilitates opening and closing without destruction of container or forms;
- clearly labelled specimens;
  package addressing.

Special attention should be paid to the mailing container, the inner container should be secure; furthermore the outer container should contain sufficient porous material to accommodate all the liquid material in case of any damage of the inner container; re-use may reduce costs.

2.3.5 Forms

Forms for the collection/recording of data that accompany the specimen should be as simple as possible to reduce confusion in entering and reading data and facilitate its entry into the data bank of the EQA scheme. Laboratories should be identified only by code (e.g. by number), and space should be provided to record date of receipt and specimen analysis. If it is anticipated that the scheme will increase the number of analytes being tested per distribution or the number of participants, the forms should be designed accordingly. The constant use of a single format will reduce errors and greatly ease data recording.

The outline of a record ing form is given in Appendix A.

2.3.6 Data analysis and reporting

These aspects of the scheme and reporting procedure are addressed in Section 4 and Appendices B and C.

2.4 Designing an EQA scheme

EQA schemes may have different designs in order to serve different purposes, for example:

Type 1. EQA designed to document current analytical performance to a third party, e.g. authorities, accreditation bodies, or the public (not the primary target of these guidelines).

This type of scheme ideally requires the expected value to be completely unknown to the participants. This may be achieved by using a selection of codes to identify a given sample and by having different samples running simultaneously within the same scheme. Only small amounts- of serum should be available in each vial, so that multiple determinations cannot be carried out. The sample should resemble fresh human serum and should ideally be labelled in the same way as patient samples.

Type 2. Those designed to inform individual laboratories about their current analytical performance.

The requirement for doing the check in a "blind" manner is basically the same as in type 1. However, because there are no external consequences ("punishment") for bad results, it is unlikely that the laboratory would analyse the sample differently from routine work. Therefore the identity of the sample need not be hidden. Education of participants so that they understand the importance-of doing the analyses on these samples in the same way. as usual patients samples will further decrease the need for hiding the identity of the sample.

Type 3. Those designed to support quality improvement by evaluating different sources of error, e.g. at different concentration levels, random error, systematic error, and certain types of nonspecificity.

This type of EQA scheme is intended for supporting the laboratory by revealing the causes of errors. The evaluation requires a variety of control samples. Several measurements obtained on the same sample in different runs are essential in order to obtain statistically sound

information. For suitable data presentation consult Appendix C. In this type of EQA scheme "blind" controls are probably unnecessary.

If only one sample is circulated in schemes of type 1 or 2, the user cannot know whether an erroneous result is caused by imprecision or bias. Two samples at different concentration levels may provide more useful data. Type 2 schemes provide more informative data especially if the same specimens are measured on several occasions and cumulative reports are prepared. The last type of scheme is most helpful to the individual laboratory and provides information which is easy to understand. However, it requires several materials and measurements, and it is probably difficult to operate consistently in a developing country. In many cases schemes are used for a combination of these purposes.

3. MATERIALS

3.1 Background for selection and preparation of sera for EQA schemes

Control serum should resemble fresh human serum as closely as possible in order to avoid interference from the matrix or other components. Although in principle the use of human serum has advantages, its use is only recommended when animal serum is inappropriate, for example, methods involving the use of antibodies against human protein or when major matrix effects are evident. Ethical consideration may prevent use of human donors for supplying material for analytical control if blood is required for clinical purposes and because of the risk of human serum incorporating agents that cause infection (for example, hepatitis, AIDS, etc.) The organiser needs to balance the cost, availability and risk in the choice of serum for EQA.

All control sera (human or animal origin) must be stable. Stability may be achieved by sterilisation (filtration), by the addition of chemicals or by lyophilisation.

In preparing EQA serum samples a range of concentrations are needed, see Table 3.

A recommended low cost procedure involves stabilising serum with ethanediol (Appendix F). Lyophilisation produces a dry material that is stable for several years when stored correctly, it is however expensive to produce and requires reconstitution with an exact volume of water. Lyophilised bovine serum (see Appendix E) has been widely used in low cost quality control schemes because of its availability and robust lipoproteins. There are some matrix interferences but for those components shown in Table 3, there are few problems with bovine serum stabilised either with ethanediol or by lyophilisation, although total protein and albumin show small matrix interferences. Electrode assemblies in direct ISE instruments may be damaged by ethanediol.

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Table 3. Examples of desirable concentrations of analytes in sera for EQA scheme.

Analyte in blood or plasma	Recommended range* of concentration of components in the control sera	Typical reference range in blood or plasma
Alanine aminotransferase	20-125 U/L	< 42 U/L
Alkaline phosphatase	40-250 U/L	20-90 U/L
Amylase	200-700 U/L	70-340 U/L
Aspartate aminotransferase	20-125 U/L	< 42 U/L
Total bilirubin	20-200 umol/L	2-21 umol/L
Calcium	2.00-2.90 mmol/L	2.25-2.60 mmol/L
Creatinine	80-500 umol/L	60-130 umol/L (male) 40-110 umol/L (female)
Urea	3-20 mmol/L	3.3-7.7 mmol/L
Glucose	2.5-25 mmol/L	non-fasting 3.5-7.4 mmol/L fasting 3.6-6.4 mmol/L
Potassium	3.0-6.0 mmol/L	3.5-5.2 mmol/L
Total protein	40-90 g/L	60-80 g/L
Albumin	20-55 g/L	30-45 g/L
Sodium	120-160 mmol/L	135-l 46 mmol/L

*The recommended concentration range of the analyte in the control sera is appropriate to methods with the reference range shown

3.2 Acquisition of materials from industry

Possible sources of materials or specimens for distribution to scheme participants are the commercial suppliers of quality control products to laboratories. However, it is important to establish that the approach is not meant to undermine the sales activities of the companies concerned. Requests should be realistic and when possible, products from several different companies should be sought.

As the prime aim of most EQA schemes is to determine between-laboratory variation the key criterion that must be met is between-specimen variation. For most analytes this can still be met with material that is approaching, or has passed, the expiry date placed on the product by the manufacturers, or by government regulations. If there is doubt about the homogeneity, the between-specimen variation should be investigated for any analyte before the circulation of samples. Careful observation of the distribution of between-laboratory results for a particular batch of control sera in relation to earlier experience may confirm the homogeneity or give clues to deterioration.

appropriate requests are made to companies for the provision of such materials, accompanied by a clear statement of the intended use, then it is possible that products may be donated, or provided at significantly lower cost. In such cases, it is appropriate to provide the sponsor with detaiis of the results obtained in the surveys using their material, as this may aid them with information regarding stability.

Requests for aid of this type should be made to directors of marketing and/or sales groups in companies operating (or contemplating operation) in the regions concerned. Advice on making approaches to industry can also be obtained from the IFCC Committee on Analytical Quality, see section 5.5. and D2 in Appendix D.

3.3 Establishing acceptable limits and target values

In an EQA scheme, results submitted by individual participants are assessed and compared either against a target value assigned by the organizing or reference laboratory or more often assessed against the consensus value ("trimmed mean value") derived from all results submitted by participants in the scheme for that analyte.

The value (or range of results) against which comparisons are made must be as reliable as possible or participants will lose confidence in the scheme.

Practical experience has shown that the consensus value usually agrees closely with the "true value" in schemes with a large number of participants but it may not be valid in schemes involving small numbers of laboratories. It is not a valid approach where a large proportion of participants have a significant analytical bias.

The consensus value ("trimmed mean") is the mean of all results after removal of outliers that will distort the distribution and hence 'affect the mean. A choice must be made between the overall consensus value and the method related consensus value. In most cases it is preferable to use the method related consensus value (essential for enzyme activity assays), although for some analytes and methods there should be no significant difference between them (e.g. glucose oxidase and o-toluidine methods for plasma glucose). However the extent of the agreement between method related and overall consensus values depends on the methods in use and the type of control material.

A general approach to calculating method related consensus values is as follows:

1   Organise the data by analyte and by methods; preferably groups of more than 20 results, and avoid forming groups of less than 10 results;

2   For small schemes, scrutinise the data and exclude any results that are obviously wrong;

3   Calculate the mean and standard deviation (SD) for each group;

4   Reject any result outside the range +/- 3SD; (use +/- 2SD if n = less than 20)

5   Recalculate the mean and SD for the remaining values;

6   Repeat steps 4 and 5 until all values fall within the range determined in step 4.

7   The mean of this last group (ie with no outliers) is the method related consensus value.

Acceptability criteria .

Many EQA schemes use the "state of the art" approach for describing "acceptable limits". Having calculated the method related consensus value, the acceptable limits may then be set to the range encompassed by +/- 2 CV% (mean +/- 2SD). It may be apparent that because of poor performance, the SD exceeds recommended or attainable levels (Table 4). In such cases, and by agreement with professional colleagues, the acceptable limits may have to be narrower than mean +/- 2SD; however, local analytical capability must by considered so that unachievable limits are avoided   See also section 5.2, Table 4, and D2 in Appendix D.

Limitations of the consensus approach

The procedure described will identify and exclude outliers but it will not separate two different populations of data (e.g. from two methods with different means). To identify such problems the data must be displayed as a histogram looking for deviation from a normal distribution, e.g. bimodal distribution which may be the case for data derived from two different methods.

Validating the consensus value

The consensus value may be validated by analysing the distributed control material by reference methods although this should only be attempted in laboratories with adequate training and experience of such methods. Some reference laboratories in Western Europe or North America may be willing to provide   assistance.

The routine methods used by participants in the scheme may also be used but the laboratories assigning the values must be confident of their bias and precision. Recovery studies can be helpful here. An alternative approach is to compare the consensus values obtained for the same control material from different schemes in for example different countries or regions. It may be possible to obtain limited quantities of materials which have been distributed in EQA schemes in Western Europe or North America to assist in scheme validation.

4. EVALUATION

There are numerous styles and formats used for presenting results of EQA schemes. Many of the well established schemes of Western Europe and North America are relatively complex and reports are produced by computer from large data banks. Common to all, however, is a requirement that participants must easily confirm their submitted values and that interpretation of their performance is clear and concise.

Developing schemes are recommended not to attempt incorporation of algorithms that assess overall, or long term performance, unless clear instructions are provided that facilitate understanding by all participants. It is far more important to provide rapid information that clearly illustrates, for each analyte being studied, the deviation of the laboratory's results from the overall consensus mean and from the appropriate method (or instrument) consensus mean. As an alternative to the usual units, the deviation from overall or method mean can be expressed as number of standard deviations or as the percentage of laboratories achieving results within the acceptable interval.

Report(s) produced must communicate the following:
- confirmation of submitted value(s);
- comparison of result(s) with all participants (often depicted by use of histograms);
- comparison of result(s) with relevant method/instrument mean (often depicted by use of histograms);
- clearly illustrate the performance limits established for the scheme;
- recommend that laboratories demonstrating unacceptable performance take corrective action, or seek advice;
  provide information on long term performance, or trends, for each analyte when appropriate.
  Note: This information can be provided in a separate report or periodically.

Some example reports are provided in Appendix C.

5. EDUCATION

5.1 Introduction

EQA schemes generate a large amount of data which can be used by scheme organisers to assist in the education of participants and potential participants. Perhaps the most important aspect is that frequently EQA data can be used to convince colleagues about the need to introduce or improve quality assurance including IQC. EQA alone is unlikely to yield improvements in the absence of effective quality assurance and IQC.

In designing the EQA scheme and the associated documentation, consideration must be given to the degree of interaction between organizers and participant. Will the organizer, for example,

- report back statistical data only?
- assist in the interpretation of data?
- advise on changes of calibrators or methods when consistent bias is detected?

An interactive programme is likely to be more effective in improving quality, particularly where there is not a high level of training for laboratory staff. EQA organisers must accept an educational role and this section of the document and the material in the Appendices are designed to assist them in that function.

Some basic educational information can be included with participants' reports - for example, how to compare an individual result with the target value and the "acceptable interval", etc. It may be possible to design some distributions to provide particular educational information; for example, the quality of calibrators has been a problem in many countries and the distribution of a stable calibrator (as a sample) and' an EQA sample together permits an assessment of the reliability of the participants' calibrator for the analyte of interest. Another example is the circulation of photometer controls such as solutions of a dye or a chemical.

A further educational benefit of EQA data is that analytical methods with poor performance can be identified and participants encouraged to use recommended methods, however, the possible contribution of matrix effect to poor performance should not be overlooked.

Workshops can be of great benefit in assisting individuals from participating laboratories, and should include the following:

- the importance of quality assessment;
- methods of quality assessment;
- preparation of calibrators;
- definitions and basic statistics in quality control;
- interpreting IQC and EQA results and resolving problems;
- preparation of QC materials;
- pre- and post-analytical factors that affect the quality of patients' results.

In some countries, the use of video film may be possible, but whatever educational method is used, there must be time for audience participation. Furthermore the course must be appropriate to the audience, to the culture, and to the existing analytical capability. It is' important that institutions and individuals recognize the significance of quality assurance in all aspects of laboratory operation. At the same time it should be emphasized that successful application of quality assurance procedures can be achieved at low cost.

5.2 Desirable performance standards

This document is based on, and recommends, performance standards based on analytical variation. Other systems, based on biological variation or clinical strategies are not recommended during the early stages of an EQA scheme.

Table 4 provides an elementary guide and examples of the performance standards that are observed in EQA schemes in developed and developing countries. It is recommended that the values achieved by established EQA schemes in the developed countries (column 1) be employed as an index of proficiency; **note,** lower values may be achieved.

Table 4. Examples of performance standards*

Analyte in blood or plasma	Performance (CV%) in Developed Countries	Performance (CV%) in DEVELOPING Countries	
		Early stage of EQA schemes	Established EQA** schemes (> 10 years)
Alanine aminotransferase	9	25-75	10
Alkaline phosphatase	7	25-75	10
A m y l a s e	4	25-75	-
Aspartate aminotransferase	6	25-75	10
Creatinine	5	12-20	10
Urea	5	15-23	8
Glucose	4	8-18	7
Total bilirubin	8	15	12
Calcium	3	8-15	6
Potassium	3	6-8	5
Sodium	1.5	4	2
Total protein	**3**	**4-1**0	**5**
Albumin	**5**	**6-1**0	**6**

* Values are dependent on concentration and matrix of control sample, selection of methods included in the calculation, and procedures for eliminating outliers which differ from scheme to scheme.

** Data provided by EQA scheme of the Association of Clinical Biochemists of India.

5.3 Statistical procedures

Some EQA schemes have never proceeded beyond the planning stage because of the belief that a computer is required to cope with the statistical evaluation of the data. The basic statistics however, are not difficult and even quite large schemes can be managed using a scientific calculator. Similarly, IQC data can be assessed and analysed using a hand held scientific calculator.

The statistical component of an educational programme, should reflect what participants need to know in order to:

- interpret EQA data;
- understand, implement and interpret IQC.

Bias and imprecision must be defined and the statistical methods for assessing them explained. The concept of a "Gaussian" ("normal") distribution and the calculation of mean, standard deviation and coefficient of variation should be described using examples from the participants' daily life experience when appropriate, before moving on to examples from laboratory measurements. The use of calculated indices to assess variation in EQA schemes should be explained.

In describing the assessment of control data it is essential to start from the perspective of "What is acceptable performance?" Too many laboratory workers have wrongly understood that the mean +/- 2SD always represents acceptable performance, regardless of the extent of the variation. Examples of the definitions and statistical calculations are given in Appendix B.

5.4 Trouble Shooting

Any educational material or training programmes must include practical information on trouble shooting and on the interpretation of EQA (and IQC) data, to enable participants to distinguish poor performance due to imprecision and other factors from that due to bias. A plot showing the results (y) against target values (x) will often be helpful in illustrating problems.

5.5 Help and advice

Help and advice to scheme organizers may be obtained through a request to the current chairman of the Committee on Analytical Quality under IFCC's Education and Management Division or to the organizers of the appropriate WHO International EQA scheme, who may also be able to advise on training etc. to assist with establishing the scheme; see D2, D3, and D4 in Appendix D.

ACKNOWLEDGEMENTS

NOTE: This document utilizes conventional definitions, nomenclature, and style. The readers, however, are encouraged to study recommendations issued from ISO, IFCC and IUPAC where definitions and nomenclature of a more systematic nature are found; see D2, and D5 in Appendix D.

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APPENDIX A. HANDLING OF EQA SAMPLES IN THE PARTICIPATING LABORATORY AND SUBMISSION OF RESULTS

AI. HANDLING OF EQA SAMPLES. INSTRUCTION FOR LYOPHILIZED   CONTROL SERUM.

The unopened vials of lyophilized control serum should be stored in a refrigerator. For long term storage -20 ^{0}C is advantageous. The following instructions illustrate the type of procedures and precautions necessary for reconstituting and storing control serum:

- ensure that the lyophilized material (the "cake") is at the bottom of the vial;
- remove the metal seal;
- release the vacuum in the vial by slowly removing the stopper so that the lyophilized material is not blown out when the vial is opened;
- add 20,0 ml (or 10,0 ml or 5,00 ml or 1,00 ml as appropriate) distilled water at room temperature with a certified bulb pipette*;
- cap the vial and keep in the dark at room temperature for 15 min.;
- mix carefully by inverting the vial 20 times;
- ensure visually that all the lyophilized material is dissolved;
- store in a refrigerator in the dark.

Note: The accuracy of the volume of water added should be checked by weight.

A2. OUTLINE OF A RECORDING FORM FOR SUBMISSION OF ANALYTICAL DATA.

An example, partly filled in

XXXXYYYY EXTERNAL QUALITY ASSESSMENT SCHEME

Laboratory number 0358	Date Analyzed: / /	Date of Receipt of specimen: / /

ANALYTE	METHOD CODE	CORRECT Y/N	NEW METHOD CODE	RESULT	UNITS
SODIUM	4				mmol/L
POTASSIUM	17			●	mmol/L
CHLORIDE	22				mmol/L
UREA	32			●	mmol/L
GLUCOSE	9			●	mmol/L

Storage requirements:	Room Temp	✓
	4ºC	

Note: it is imperative that instructions for specimen treatment and analysis are followed correctly – SEE INSTRUCTIONS FOR EQA SCHEME PARTICIPANTS

Deadline: Return the results to the below EQA scheme organizer before: /   /

ENQUIRIES REGARDING THE SCHEME SHOULD BE SENT TO:
        Dr. XXXXMMMM
        P.O.Box 234
        SMITHVILLE, etc.

Keep a copy in the laboratory. Filled in by:

Reserved for the EQA scheme organizer
Date of receipt of this recording form: Date Stamp

APPENDIX B. STATISTICAL METHODS FOR ASSESSING DATA

B1 Basic statistical calculation in quality control

All laboratory measurements show some variation or "error". The greater the variation, then the wider will be the spread of results around the average value. The statistical calculations that are used enable the variation to be described and thus compared with other sets of results.

There are three basic statistical terms which are used to describe the measurement variations encountered in quality control. These are:

- The average or mean value ($\bar{x}$) this is the sum of the individual results in a series of measurements divided by the number of results. The mean is thus the best estimate representing that series of results. It is expressed in the same units as the individual measurements. A reference method may provide an estimate of the "trueness", often named the conventional true value. The difference between this and the obtained mean value ($\bar{x}$) is called the bias;

- the standard deviation (SD): any series of measurements will be spread around the mean value. The standard deviation describes the spread or distribution of results around the mean value. Like the mean, SD is described in the same units as the individual measurements

- coefficient of variation (CV): this is the SD expressed as a percentage of the mean so that the spread of different series of results can be compared even though the mean values may be very different.

These three statistical terms are used to describe and compare the imprecision and bias of analytical methods within a laboratory when methods are being assessed, for routine within-laboratory quatity control and for comparing performance between groups of laboratories in EQA schemes.

Calculation of the mean, SD and CV

The example here is for a series of 15 albumin measurements made on the same sample:

- list the individual results (column x_i in Table B1)

- to calculate the mean: add up all results in column x_i to obtain the sum of x_i, ($\Sigma\ x_i$); divide this value by the number of results (15)

$$\text{mean } (\bar{x})\ \frac{516}{15} = 34.4 \text{ g/L}$$

- for each result, calculate $(x_i - \bar{x})$ and enter in the column marked $(x_i - \bar{x})$;

- calculate the square of $(x_i - \bar{x})$ ie, $(x_i - \bar{x})^2$

- divide the sum of $(x_i - \bar{x})^2$ for all results by the number of results less one (n - 1), which is 14, thus:

$$\frac{29.6}{14} = 2.1;$$

- SD is the square root of 2.1, which is 1.45 g/L;
  $\sqrt{2.1} = 1.45$ g/L
- CV is SD multiplied by 100, and divided by the mean,

Thus: $CV = \dfrac{1.45 x 100}{34.4} = 4.2\%$ Note: x is used here for multiplication

Table B1: Results x_i and calculations

	x_i	$(x_i - \bar{x})$	$(x_i - \bar{x})^2$
1	33	-1.4	1.96
2	35	+0.6	0.36
3	32	-2.4	5.76
4	36	+1.6	2.56
5	35	+0.6	0.36
6	34	-0.4	0.16
7	33	-1.4	1.96
8	35	+0.6	0.36
9	36	-1.6	2.56
10	35	+0.6	0.36
11	34	-0.4	0.16
12	35	+0.6	0.36
13	32	-2.4	5.76
14	34	-0.4	0.16
15	37	+2.6	6.76
Sum	$\Sigma\, x_i = 516$		$\Sigma(x_i - \bar{x}) = 29.6$

B2. Systems for evaluating EQA scheme data

Control-rule system

The simplest systems for evaluating EQA-schemes are based on pass/fail rules similar to those employed in internal quality control. They require definition of performance standards, and for each type of control sample, a target value and acceptable limits. The set of control rules should be optimized in such a way that the probability of error detection is high and the frequency of false alarm is low. The type of control rule which is applied should indicate the type of error. This is particularly true when more than one result is validated. Repeated application of an EQA -scheme may assist the laboratory in improving its proficiency, and may also provide opportunity for education. Each time an alert is operated, the laboratory should consider what steps it should take to improve its analytical quality.

Theoretically, the control rule system is easy to understand and should fit well with usual analytical work in the laboratory. However, to date there has been little application of sophisticated sets of control rules.

When using the control rule system, some information about uncertainty may be lost, but the EQA-scheme data collected in a laboratory over a long period will allow for independent calculation of estimates of bias and random error, so long as the target values are traceable to a reference method and a reference material. Only relative bias can be detected if there is a lack of traceability.

Scoring systems

The basic concept of most scoring systems is the expression of an individual EQA result as the deviation (d) from the target value, divided by the standard deviation (s).
This is termed the z-score

Formula A $\quad z = \dfrac{d}{s}$

Often the standard deviation (s) used for this purpose is provided by the EQA-scheme. In this case it reflects a "between laboratory" deviation. Typically, the score is -2 to +2 when based on 95 % of all results (i.e. $\pm 2\ SD$) or -3 to +3 when based on 99,9 % of all results.

Other ways of establishing the standard deviation (s) can also be relevant. One method frequently used is to select a value for "s" that is constant for a given analyte. This permits convenient assessment and comparison of all participants; the use of this method in the WHO International EQA scheme is described below.

In surveys using multiple samples (n), the outcome of the survey can be assessed by determination of the average of the z-scores.

Formula B $\quad [\Sigma(z)]/n$

Note that in Formula B all z-scores should be treated as positive. Ideally the value of s should be derived from clinical requirements or analytical goals but a constant based on the state of the art can be useful.

Alternatively, the scores may be combined as variances. However, the outcome will be dependent on the number of observation.

Formula C $$\sqrt{\dfrac{\Sigma \ (z^2)}{n-1}}$$

Combination of scores with time for multiple surveys or even for multiple analytes is possible. These types of calculations are included in the ISO/IUPAC/AOAC   guide on EQA schemes (5). The guide acknowledges the utility of combined scores but warns of the potential for misinterpretation or abuse by inexperienced   users.

An example of a scoring system which has been applied widely, including WHO's International EQAS, is Variance Index. Here the z score uses a constant s term, based on an assumption of a constant CV (derived from state of the art) across the concentration range assessed. The score is multiplied by 100 to yield a Bias Index Score (BIS) as a scaled deviation from the target which is limited to +/-400 by truncation of extreme   deviations.

Cumulation over time (typically 6 months or 10 scores) of the BIS and Variance Index Scores (VIS; the absolute value of BIS, ignoring its sign) yields three performance indicators for each analyte:

Accuracy (total error)	MRVIS Mean Running VIS
Trueness (bias)	MRBIS Mean Running BIS
Consistency of bias (variability)	SDBIS Standard Deviation of BIS

Contributions to variability come from several factors (eg, concentration-dependent bias; time-related changes in bias, specimen-dependent (matrix) effects) in addition to imprecision.

The use of an s term derived from the state of the art also permits cumulation across analytes yielding an Overall Mean Running VIS (OMRVIS) based on all the laboratory's results over (typically) the previous 6 distributions. This may be used as a practically useful indicator of the laboratory's general standard of performance and of changes with time.

Scoring systems such as this have been extremely useful in many national and international schemes and are not demanding to apply. However, it is essential to note that:

- it is possible for a satisfactory OMRVIS to conceal unsatisfactory performance for one or more individual analytes, and assessment should not be limited to an overall score.

- a full assessment for an analyte showing performance difficulties must include examination of the relationship between individual resuls and target values (preferably graphically), not just appraisal of MRBIS and SDBIS. Non-analytical (eg, transcription or transposition) errors and failure to return results should be assessed separately from analytical performance.

APPENDIX *C. METHODS FOR REPORTING TO THE PARTICIPANTS.*

The style and complexity of the report to participants will depend upon the facilities available to the organizers, particularly the accessibility of computer programmes for data handling and graphics. However, valid data interpretation is possible using a handheld calculator able to compute mean and standard deviation.

The minimum data reported to participants must include: the number of laboratories in the method group, the mean value (or target value) and SD of the submitted results, the results submitted by the participant, the acceptable limits, and possibly some type of score. Each laboratory can thus compare their result with the mean (or other target value) and distribution of all results obtained using that method. Comments on the results should be given when they are outside the acceptable limits; the first comment could be: "check the value"; when there are frequent deviations the comments could be "analytical problems, contact the EQAS organizer".

The use of a computer allows histograms to be produced which. assist in the comparison of an individual result with all results submitted.

Examples of report formats are shown in C1, C2, C3, C4 and C5.

C 1.   *AN EXAMPLE OF A REPORT WHICH MAY BE PREPARED WITH A HANDHELD CALCULATOR AND A TYPEWRITER.*

ANALYTE	METHOD USED	n	MEAN	SD	YOUR RESULT	ACCEPTABLE LIMITS	SCORE
Glucose (mmol/L)	All	303	8.4	1.2			
	GOD-POD	221	8.2	0.9			
	o-toluidine	49	9.1	1.0	9.4	7.3 10.9	33
	Folin & Wu	32	8.4	1.1			
	Other	1	9.5				
Urea (mmol/L)	All	284		3.2			
	DAM-TSC	140	20.0	3.0			
	Urease-Berthelot	112	18.3	2.9	23.2	16.1 - 20.5	446
	Urease-GLDH	32	18.1	2.9			
Calcium (mmol/L)	All	208	2.47	0.25			
	CPC-AMP	35	2.42	0.15			
	CPC-DEA	49	2.39	0.16			
	EDTA-titration	121	2.52	0.20	2.44	2.27 - 2.77	-63
	Other	3	2.70	0.35			

C2   *AN EXAMPLE OF REPORT FOR COMPARISON OF RESULTS FROM THE INDIVIDUAL LABORATORY WITH THAT OF ALL LABORATORIES.*
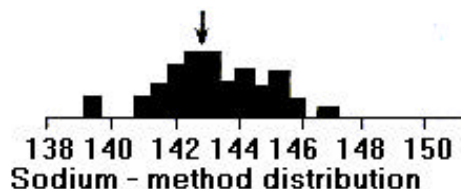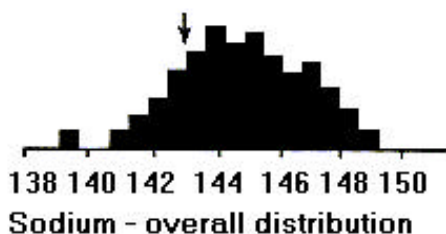
XXXXYYYY External Quality Assessment Scheme

Report for laboratory   0233   Reporting date   12/02/96
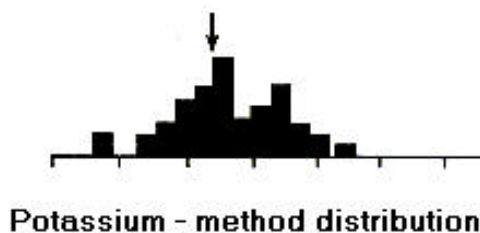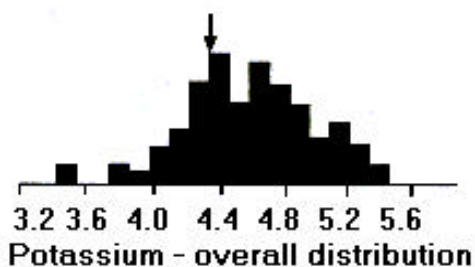

Analyte: Sodium   **Your Value: 143 mmol/L**

Overall mean	Nº	Std. Dev.	Your SD Diff.
144.2	245	2.1	-0.57
Method mean	**Nº**	**Std. Dev.**	**Your SD Diff.**
142.9	28	1.4	-0.07

Sodium - overall distribution

Sodium - method distribution

Analyte: Potassium   **Your Value: 4.3 mmol/L**

Overall mean	Nº	Std. Dev.	Your SD Diff.
4.43	241	0.75	-0.17
Method mean	**Nº**	**Std. Dev.**	**Your SD Diff.**
4.31	27	0.33	-0.03

Potassium - overall distribution

Potassium - method distribution

Histogram: Your value is indicated by an arrow.

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C3. *AN EXAMPLE FOR COMPARISON OF RESULTS FROM THE INDIVIDUAL LABORATORY WITH THAT OF ALL LABORATORIES, THE REFERENCE METHOD VALUE AND THE ACCEPTABLE INTERVAL.*

VVVVYYYY External Quality Assessment Scheme

Report for laboratory **0238**   Reporting date   12/02/96

_____

Analyte: Sodium   **Units: mmol/L**   **Your Value: 143**

Overall mean	Std. Dev.	Nº	Acceptable %
144.2	2.1	245	73

Reference
method value: 143.1

Method mean	Std. Dev.	Nº	Acceptable (%)
142.9	1.4	28	92

Acceptable
interval: 140 - 146
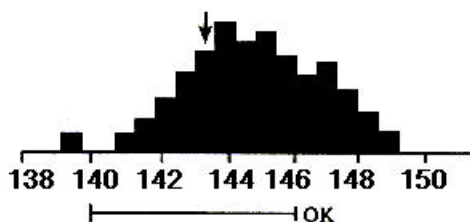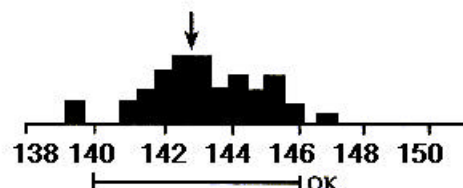
Sodium - overall distribution   Sodium - method distribution

Analyte: Sodium   **Unit: mmol/L**   **Your Value: 4. 30**

Overall mean	Std. Dev.	Nº	Acceptable %
4,43	0,75	241	73
Method mean	Std. Dev.	Nº	Acceptable %
4,31	0,33	28	92

Reference
method value: 4,45

Acceptable
interval: 4,00 - 4,90
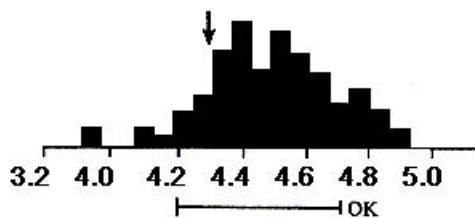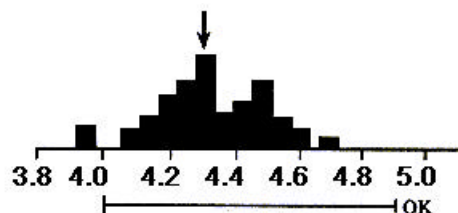
Potassium - overall distribution   Potassium - method distribution
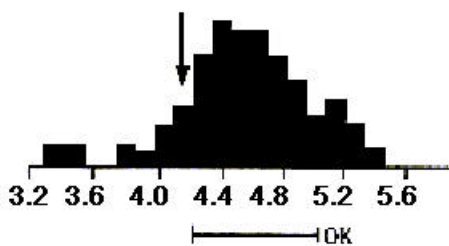
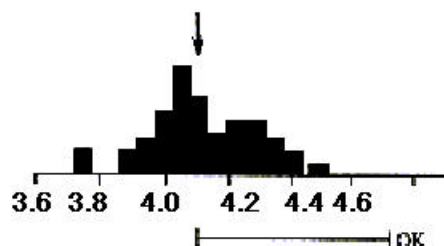**Analyte: Urea**   **Unit: mmol/L**   **Your value: 4,1**

Overall mean	Std. Dev.	Nº	Acceptable %
4,4	0,75	241	65
Method mean	Std. Dev.	Nº	Acceptable %
4,1	0,33	27	48

Reference
method value: 4,5

Acceptable
interval 4,1 - 4,9

Urea - overall distribution   Urea -method distribution

Histogram : Your value is indicated by an arrow

The data presentation shown here, Fig C4.1, is designed to provide as much useful information as possible from a series of EQA data in order to assist in trouble shooting. The presentation is suited to an EQA scheme where established target values are available. The plot uses concentration of the target value as x-axis, and the deviation from the target as y-axis showing the systematic error (bias plot). The plot typically accumulates EQA data from many series. The plot may be prepared by each participant manually or produced centrally on a computer.
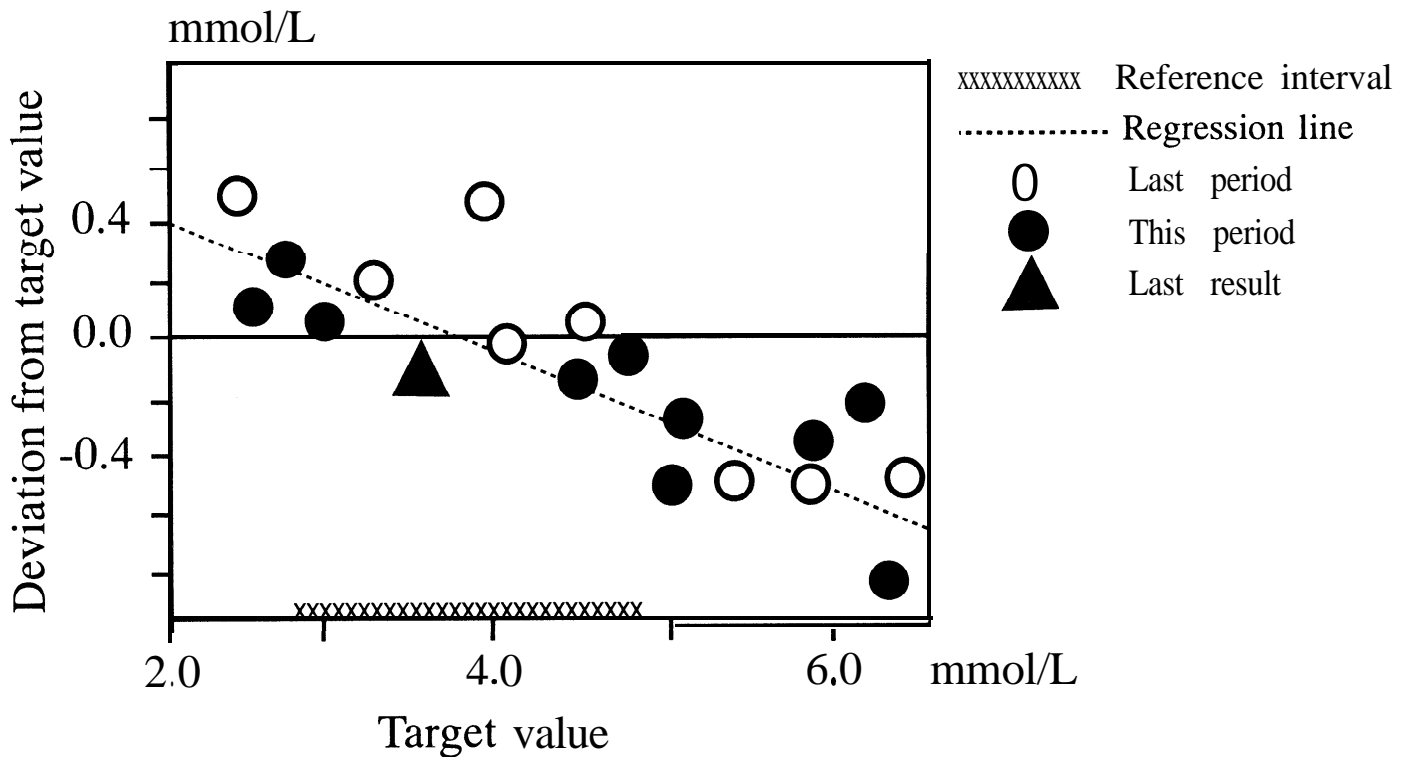

Fig. C4.1. The figure shows a dotted line which is the regression line calculated by the computer. Slope (b) and intercept of the regression line are key parameters. The plot shows as an example a systematic error of 0.4 mmol/L minus 9% (b = -0.09) of the target value. Ideally each value should be zero. The plot may be supplemented by another bias plot showing the results in historical sequence.

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C5.   EXAMPLES OF PRESENTATION FOR THE INDIVIDUAL LABORATORY OF CONCENTRATION DEPENDENT DATA SET.

Example of a survey for glucose. The same samples are spiked with different, and known amounts of glucose; the set of samples are then measured daily over 6 days. The data are calculated and illustrated graphically for the various concentrations; the observed mean value minus the expected value is shown versus the expected value (reference method value) (see Fig. C5.2). The EQA scheme organizer could do the calculations, and could where needed make comments in the report. Laboratory NN provides reliable results. Good precision and a positive bias at the low concentration are observed for the laboratory ZZ shown in the figure; the laboratory could be advised to look for e.g. wrong blanking of instrument or non-specific reaction. The laboratory could also be advised to assess the linearity of calibration, but this may become obvious after removal of the first type of error.
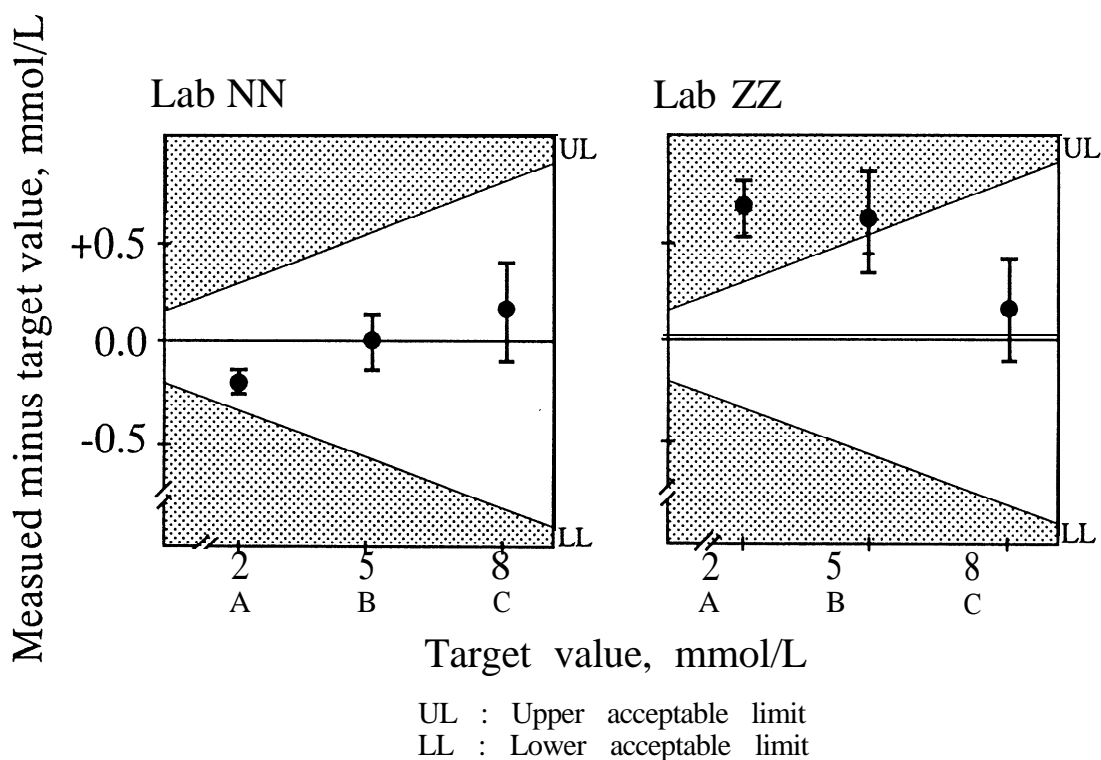

UL : Upper acceptable limit
LL : Lower acceptable limit

Fig. C5.2. Examples from an EQA-scheme. Bias plot for glucose in plasma for laboratory NN (left), which provided satisfactory* results, and for laboratory ZZ which provided unsatisfactory* results
Mean and range of 6 results are shown for each sample. The value of sample A is established by a reference method, the values of sample B and C are established by weighing (spiked samples).
* The acceptable limits were established arbitrarily for illustrative purpose.

APPENDIX D. USEFUL ADDRESSES; **how to obtain additional information**
DI.   **/FCC Technical Secretariat**
Centre du Medicament
Universite de Nancy 1
30, rue Lionnois
F-54000 Nancy, France
Fax: 33 83 32 13 22
Types of request: /FCC publications, name and addresses of national representative.

D2.   **/FCC. Education and Management Division.**
Committee on Analytical Qualify (CAQ)

Peter Hill
Dept. of Chemical Pathology, Derbyshire Royal infirmary,
Derby, DE1 2QY, United Kingdom
Fax: +44 1332 254 924

Adam U/da/l (chair)
Dept. of Clinical *Chemistry*
Herlev University Hospital
DK 2730 Herlev, Denmark
Fax: +45 44 53 53 32

Peter Wilding
Dept. of Pathology and Laboratory Medicine
3400 Spruce Street, 7103 Founders Pavillion
Hospital of the University of Pennsylvania
Philadelphia, PA 19 104-4284, USA
Fax: +1 215 662 7529

Types of request to members of CAQ: Bibliographic information, nomenclature, desirable performance standards, definitions of analytical terms and qualify terms, advice *on design of scheme, supply of control* sera and target *values (when possible), world wide contact to EQAS organizers.*

D3.   **World Health Organization (WHO)**
Unit of Health Laboratory Technology
CH-1211 Geneva *27 Switzerland*
Fax: +41 22 79 10 746
Type of request: Availability of WHO sponsored EQA schemes; registration of national or regional EQA schemes

D4.   **WHO Collaborating Center for Research & Reference services in clinical chemistry**
International EQAS for Clinical Chemistry, Wolfson EQA Laboratory,
Queen Elizabeth Medical Centre
P.0. Box 3909
Birmingham B15. 2UE, UK.
Fax: +44 12 14 14 11 79
Types of request: Participation in the scheme, advice on scoring systems.

D5.   **IUPAC-IFCC/C-QU(CC)**
Henrik Olesen
Dept. of Clinical *Biochemistry*
Rigshospitalet
Tagensvej 9
DK-2200 Copenhagen NV, Denmark.
Tel: +45 35 45 76 42
Fax: +45 35 45 27 45
Type of request: Names of quantities.

APPENDIX E. PREPARATION OF LYOPHILIZED BOVINE SERUM

A detailed description of the preparation of lyophilised bovine serum and procedures for adjusting the concentrations in the final product is given in WHO LAB/81.4 (2). The information provided below is compiled from WHO LAB/81.4 and other literature.

Collection of blood and separation of serum

Ideally, serum is obtained from manufacturers of bacteriological culture media. When this is not possible, fresh blood from a slaughter house may be used. It should be collected using as aseptic conditions as possible. The blood must be collected into dry containers.

Achieving the desired concentration

Concentrations of analytes in the serum may be adjusted by several techniques. The simplest is dilution with water to decrease the concentrations.

Calcium, sodium and potassium may be added as salts, for example, chlorides; and urea, creatinine and glucose added to increase the concentrations of these analytes (see also Appendix F). Bilirubin and uric acid can be added after dissolving in potassium or sodium hydroxide (0.1 mmol/L).

Apparent triglyceride concentrations can be increased by the addition of glycerol although such material will not be suitable for methods involving extraction. There is no simple procedure for increasing cholesterol concentrations.

Adjustment with enzymes will in general lead to method dependent final activities because the enzymes used may behave differently from normal human serum enzymes with different methods. Sterile filtered (pore size <0.2 μm) human saliva may be used to increase amylase activity. Appendix F of this document describes the preparation of placental alkaline phosphatase and also indicates alternative commercial sources.

If the desired concentrations cannot be obtained by simple dilution or addition of selected analytes, then "cryoconcentration" as described in Appendix F and in WHO LAB/81.4 may be helpful. Alternatively, for some centres ultrafiltration/dialysis using disposable kidneys may be possible.

When the serum is required for monitoring carbonate and carbon dioxide, then a special hydrogen carbonate reconstitution fluid must be provided.

Filtration and dispensing into vials

After the additions have been made, the serum must be filtered before dispensing into vials. The less contaminated the product, the better it will keep. Complete sterility is difficult to achieve and adds considerably to the expense of production. However, the best level of purity which can be achieved locally should be the goal. Turbid contaminated materials need filtration through one or more deep filters followed by a membrane filter (pore size < 0.6 μm is often sufficient).

A variety of filtration systems are available. The serum is best forced from a vessel with an oval internally fitted lid, pressurized with nitrogen gas, 10 to 25 lbs per in^2 (70 to 140 kN/m^2) through a supported pad, cartridge or membrane filter either directly or employing a cascade system, the latter reducing particle size by stages. Cognisance must be taken of the total volume of serum to be filtered

per batch and the fact that serum is more difficult to filter than simple aqueous solutions. Small losses occur during this stage e.g. absorption of bilirubin onto filter material.

For dispensing supplies of vials, stoppers and aluminium closure rings are required. The vial for the lyophilized serum should have a total volume 2,5 times that of the serum dispensed, e.g. 25 ml vials are required for 10 ml serum and 50 ml vials when 20 mL are dispensed. Amber glass vials are preferable to clear glass.

Each batch of serum must be thoroughly mixed before dispensing starts. For repetitive deliveries an automatic dispenser capable of a consistent precision of 0.2 % or better of the volume dispensed should be used. Because of the proteinaceous nature and dissolved gases in serum, difficulty is experienced with certain syringe type automatic dispensers and it is essential to check the volume delivered throughout a run in at least one tenth of the vials by weighing before and after delivery.

Records should be kept of vial positions in the filling and lyophilization processes. In this way any deficiencies in the processes can be detected.

Lyophilization

The principle and operation of a typical freeze drier is described elsewhere. A temperature probe (thermistor) is placed in one of the vials to monitor the drying process and this vial is placed at the core of each batch. The tray carrying the vials containing the dispensed serum is placed in a deep freeze and the serum frozen for a period of several hours after which it is placed in the freeze drier and the operation started with a minimun of delay. Where facilities allow, it is desirable to cap the vials under vacuum or under an atmosphere of nitrogen while still in the chamber in order to minimize problems due to oxidation. Alternatively on removal from the chamber at the conclusion of the drying process, the vials are capped immediately and aluminium closure rings placed above the rubber stoppers and compressed by a hand crimper.

Control of stability

It is essential to check the stability when establishing the production of control sera and every time changes are made to the production procedure. Alanine aminotransferase and glucose should be studied because they are the most vulnerable components. Bilirubin may also be included. Complete stability of the components in the sera is not achievable and the stability may vary from one batch of control serum to another.

The checks should be done on the lyophilized product stored at 4 ^{0}C and at 25 ^{0}C over its life time. A study of stability at 35 ^{0}C e.g. for one month, is also relevant in hot countries to simulate mailing conditions. Confirmation of stability may be achieved by the comparison of consensus mean values obtained on repeated testing of the batch in the same region.

Check of homogeneity

Initial test. Vials to test for homogeneity should be selected so that the extreme conditions are covered. The first, the middle, and the last vial in the filling line should be selected as well as two vials from the middle and two from outer positions of the lyophilization chamber. A sample from each vial is measured three times within one series of analysis ensuring that all vials will be equally affected by any possible

drift with time in the analytical system. The following components should be checked: sodium, calcium, total protein, bilirubin, glucose and alanine aminotransferase. Analysis of variance may be used to show whether a significant between-vial variation is present.

Confirmatory investigation. If the initial test indicates a significant between-vial variation this may be confirmed by a more complete investigation covering one or two percent of all vials and of one or more critical components, such as glucoses and alanine aminotransferase.

Preparation of labels

Labels are prepared which carry explicit information of the name of the manufacturing laboratory, date of manufacture, batch number, the type of matrix, the recommended storage conditions and where appropriate the volume of distilled water (or other reconstitution fluid) to be added, together with any other relevant information.

Storage

Lyophilized sera should be stored in a refrigerator within the temperature range 2-8 ^{0}C Storage at -20 ^{0}C improves long term stability.

Distribution

Lyophilized material can be transported throughout the world if carried as air freight and immediately stored under refrigerated conditions on arrival at its destination. Where ambient temperature is high, delay can result in lowering of certain assigned values particularly glucose and alanine aminotransferase.

PREPARATION OF STABILISED LIQUID QUALITY CONTROL SERUM
TO BE USED IN CLINICAL CHEMISTRY

by

Mr. D M Borwning, Laboratory Manager
Department of Clinical Chemistry
Wolfson Research Laboratories
Queen Elizabeth Medical Centre
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Health Laboratory Technology
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CONTENTS

SUMMARY

The use of Internal Quality Control (IQC) and External Quality Assessment (EQA) to continuously monitor laboratory performance are accepted as essential parts of the function of any health laboratory.

Laboratories in many countries are disadvantaged by virtue of the non availability or high cost of commercial quality control sera. The WHO document (LAB/81.4) was prepared to encourage local production of quality control materials to ensure their availability. Guidelines for the preparation of lyophilised and liquid control sera are given in that document.

This publication extends document LAB/81.4 by describing the preparation of liquid control sera stabilised with ethylene glycol (ethanediol) which expands the suitability of the material to include the control of commonly measured enzymes in addition to other analytes. The material described in this document can be used for IQC and EQA of the clinical chemistry methods described in "Methods Recommended for Essential Clinical Chemical and Haematological Tests in Intermediate Hospital Laboratories" (LAB/86.3).

1   MATERIALS FOR EVALUATING LABORATORY PERFORMANCE

IQC and EQA are required in all laboratories in developed and developing countries. They involve the use of analytical results obtained when suitable materials, usually serum, are analysed in the same way as specimens from patients. The results are used (i) to decide whether a batch of tests is acceptable and can therefore be reported to the requesting doctor (IQC) and (ii) to observe analytical comparability between laboratories within a region, country or internationally (EQA).

The control materials used for these purposes must be stable; this has been achieved in the following three ways:

(1)   Liquid serum can be stabilised by sterilisation (filtration).

(2)   Liquid serum can be dispensed into ampoules and then lyophilised.

(3)   Liquid serum can be stabilised by the addition of chemicals.

1.1   Stabilisation by sterilisation

This method produces serum which contains no viable bacteria and which is stable at 4^0C for several weeks. Serum prepared in this way is stable for 2-3 weeks at $20\text{-}25^0C$ but is unstable in warmer climates. A major problem with the preparation of this material is the provision of a suitable sterile environment and of dry sterile bottles.

1.2   Stabilisation by lyophilisation

This method produces a stable product which is widely used in more developed countries. Serum is filtered and then lyophilised. In its dried state it is stable for several years when correctly stored. There are several disadvantages to this method, viz:

(a)   The serum must be accurately and precisely dispensed prior to lyophilisation.

(b)   It is an expensive process, requiring significant capital expenditure.

(c)   It requires considerable expertise to obtain a satisfactory product

(d)   Reconstitution of the material with an exact volume of water is necessary prior to use.

(e)   Matrix changes occur during lyophilisation and the reconstituted serum may be significantly turbid.

It is doubtful if developing countries with limited resources could afford the expense of total reliance on lyophilised material. However, there will always be a requirement for small amounts as a stable reference material. In some instances it may be more cost effective to purchase these from a suitable commercial source.

1.3   Stabilisation by chemicals

This method produces a cheap source of stable liquid serum suitable for IQC and EQA. A variety of compounds have been used to stabilise serum and serum preserved in this way will remain stable for several weeks when stored correctly. The major advantage of this method are:

(a)   Low cost.

(b)   It is a simple process requiring normal laboratory expertise.

(c)   No reconstitution is required prior to use.

(d)   Little or no obvious matrix effects on subsequent analysis by manual methods.

However, the preservatives chosen should not interfere with methods in common use. Laboratories can prepare a range of sera with different concentrations of common analytes thus enabling the laboratory worker to check the analytical process over a wide concentration range.

2   CHOICE OF STARTING MATERIAL

The use of animal sera is strongly recommended rather than human sera for the following reasons:

(a)   The serious risk of incorporating infectious human sera into the material with agents that cause serum hepatitis and acquired immune deficiency syndrome, etc.

(b)   The use of human donors to supply large quantities of general purpose control serum is not justifiable.

(c)   For the purpose envisaged in this document, viz, the quality control of most tests including those listed in LAB/86.3, the use of animal serum is satisfactory.

Bovine serum is the best source in many countries; however, in some countries its use may not be acceptable. Some alternative materials are porcine and equine although other animal sources may also be acceptable. Table 1 compares the approximate concentrations of some common analytes in human, bovine, equine and porcine serum. Details for the collection of animal blood may be found in the WHO document LAB/81.4.

TABLE 1 - APPROXIMATE CONCENTRATIONS OR ACTIVITY OF ANALYTES IN HUMAN AND SOME ANIMAL SERA (SI UNITS)*

ANALYTE	UNIT	HUMAN	BOVINE	EQUINE	PORCINE
Albumin (BCG)	g/l	43	32		
Alk Phos**	U/l	55	56		
Amylase **	U/l	180	15		
AST**	U/l	26	85		
Bicarbonate	m mol/l	25			
Bilirubin	μmol/l	7	3,0	10	26
Calcium	m mol/l	2,5	2,68	3,08	2,49
Creatinine	μmol/l	80	97	97	88
Glucose (fasting)	mmol/l	5,0	2,8	4,1	3,9
Potassium	m mol/l	4,3	4,3	4,0	4,6
Sodium	mmol/l	141	142	139	148
Total protein	g/l	70	68	68	80
Urea	mmol/l	4,7	4,3	4,7	5,0

*   The comma (,) has been used to indicate the decimal fraction as recommended in the SI for the Health Professions, World Health Organisation, 1977.

**   Measured by the methods described in LAB/86.3

3   METHODS OF PREPARING STABILISED LIQUID SERUM

It is essential that all laboratories have reasonably large volumes of stable quality control serum available. These need to be of good quality but cheap and easy to produce.

Of the methods available the one chosen must utilise cheap and widely available chemicals. Some compounds are known to invalidate enzyme assays and should not be used if the material is to be used for enzyme measurements. Thiomersal, borate, fluoride, azide and antibiotics have all been used as preservatives but have been found to be less satisfactory then ethylene glycol. In serum containing 15% ethylene glycol, all of the constituents listed in Table 2 are stable at -20ºC for at least 8 months. At 4ºC, alkaline phosphatase, AST and bilirubin are stable for 4 months and all other constituents listed in Table 2 are stable for at least 8 months. Serum containing 15% ethylene glycol is also stable at higher temperatures for shorter periods. Initial studies indicate that all of the serum constituents described in Table 2 are stable at 25ºC for six days and at 37ºC for three days. The stability at these temperatures is likely to be variable depending on the level of contamination by microorganisms. Serum prepared in this way may therefore be distributed at ambient temperature for external quality assessment programmes in addition to its use for internal quality control.

4   METHODS FOR THE PREPARATION OF SERUM USING ETHYLENE GLYCOL (ETHANEDIOL)

The addition of any liquid to serum results in a dilution of the concentration of all analytes. For many analytes this is undesirable and involves the laboratory in the addition of several compounds to restore the concentration of some constituents. To avoid this it is best to start by preparing a carefully mixed serum pool which is then frozen. On thawing, the top layer will contain very low concentrations of all constituents. A volume, equivalent to 15% of the total, is removed and replaced with the same volume of

ethylene glycol. It is then mixed carefully and thoroughly. At this stage the serum may be assayed for any constituent and compounds added to elevate the concentration of certain analytes.

4.1   Preparation of a preliminary batch

It is important that laboratories have available to them batches of quality control sera in the low, medium and high concentrations of all analytes likely to be measured as recommended in WHO document LAB/86.3. These materials enable laboratories to carry out quality control checks over a wide analytical and physiological range. However, we strongly recommend the preparation of a small practice batch with the concentration of most analytes in the medium range. This helps the laboratory gain expertise and avoids the possible loss of valuable serum. When familiar with the techniques the laboratory can prepare larger volumes at low, medium and high concentrations and this will involve more extensive additions of various materials.

Start with a batch of 250 ml;

1   Start with 250 ml of fresh bovine (porcine or equine) serum. Human serum is <u>not</u> recommended (see Section 2).

2   Carefully mix to ensure homogeneity and freeze completely at -20⁰C. Since it is important to avoid possible deterioration of this serum by allowing it to remain at ambient temperature too long, the remainder of the process <u>must</u> be completed within one working day.

3   Allow to thaw at room temperature.

4   When completely thawed carefully remove 38 ml of the top layer of fluid. This is 15% of the total volume and consists mainly of water or very dilute serum.

5   Replace this volume by adding 38 ml of ethylene glycol.

6   Mix very carefully and filter through non absorbent cotton wool to remove any large aggregates.

7   The glucose and urea concentrations will be too low for a serum of medium concentration and must be raised by adding 140 mg of glucose and 44 mg of urea. These solids must be dissolved and the preparation carefully and thoroughly mixed to ensure homogeneity.

8   The serum can then be dispensed into clean, dry, well capped bottles or vials and stored in the refrigerator.

9   Before use the serum should be removed from the refrigerator and allowed to rise to ambient temperature; mix gently and thoroughly before use.

4.2   Preparation of a medium concentration quality control serum

1   Start with 1 litre of fresh bovine (porcine or equine) serum.

2   Carefully mix to ensure homogeneity and freeze at -20⁰C. It is important to avoid possible deterioration of this serum by allowing it to remain at ambient temperature too long. The remainder of the process <u>must</u> be completed within one working day.

3   Allow to thaw at room temperature.

4   When completely thawed remove 150 ml of the top layer of fluid. This is 15% of the total volume and consists mainly of water or very dilute serum.

5   Replace this volume of fluid by adding 150 ml of ethylene glycol.

6   Mix very carefully and filter through non absorbent cotton wool to remove any large aggregates.

7   Measure the concentration of the analytes in which you are interested and construct a table identical to Table 2, filling in your own measured concentrations (column 4).

8   Fill in the desired concentrations in column 3 and use this table to calculate the amounts of analytes to be added as described below.

9   The concentration of constituents may be similar to the values indicated in column 4 of Table 2 . It is anticipated that adjustments will be required for alkaline phosphatase, amylase, bilirubin, glucose and urea.

Table 2 - APPROXIMATE MEASURED AND DESIRED CONCENTRATIONS OR ACTIVITIES FOR MEDIUM CONCENTRATION QUALITY CONTROL SERUM (BOVINE ORIGIN)

CONSTITUENT	UNITS	DESIRED CONCENTRATION*	MEASURED CONCENTRATION*
Albumin	g/l	38	38
Alk Phos	U/l	150	50
Amylase	U/l	300	0
AST	U/l	80	80
Bicarbonate	mmol/l	14	14
Bilirubin	μmol/l	35	3
Calcium	mmol/l	2,55	2,55
Creatinine	μmol/l	90	90
Glucose	mmol/l	5,6	2,5
Potassium	m mol/l	4,1	4,1
Sodium	m mol/l	135	135
Total protein	g/l	65	65
Urea	mmol/l	7,0	4,1

*   Or activity

Adjust the concentration of analytes as follows:

(a)   <u>Alkaline phosphatase</u>

Amount to be added to 1 litre =

desired activity - measured activity;

ie, from the example in Table 2;

150 - 50 = 100 units to be added to 1 litre. This may be added as a powder or a concentrated suspension.

Alkaline phosphatase is available commercially. A detailed description for the preparation of alkaline phosphatase from human placenta is given in Appendix 1.

(b)   <u>Amylase</u>

Amount to be added to 1 litre =

desired activity · measured activity;

ie, from the example in Table 2;

300 · 0 = 300 units to be added to 1 litre.

Human saliva contains about 1000 units amylase/ml, so 0,3 ml contains 300 units. Collect about 1 ml of saliva, centrifuge at about 2500 rpm for 10 minutes then add 0,3 ml of supernatant to 1 litre of stabilised control serum. Measure the amylase activity of the control serum at 37⁰C to confirm that it is approximately 300 U/l.

(c)   <u>Bilirubin</u>

Amount to be added to 1 litre =

desired concentration · measured concentration;

ie, from the example in Table 2;

35 · 3 = 32 μmol (1 μmol/l = 0,59 mg)

32 x 0,59 = 19 mg to be added to 1 litre.

Weigh out 19 mg of bilirubin (32 μmol), dissolve in 4,0 ml of 0,1 mol/l sodium hydroxide to produce a clear red solution; when the bilirubin has completely dissolved, add the solution, with constant stirring, to the 1 litre of stabilised control serum. The procedure should not be carried out in a brightly lit place and, after adding the bilirubin to the control serum, it must be protected from light to avoid destruction of the bilirubin.

(d)   <u>Glucose</u>

Amount to be added to 1 litre =

desired concentration · measured concentration;

ie, from the example in Table 2;

5,6 · 2,5 = 3,1 mmol (1 mmol = 180 mg)

3,1 x 180 = 558 mg.

Weigh out 558 mg of glucose and add it to 1 litre of stabilised serum, mix gently and thoroughly to dissolve.

(e)   <u>Urea</u>

Amount to be added to 1 litre =

desired concentration · measured concentration;

ie, from the example in Table 2;

7,0 · 4,1 = 2,9 mmol (1 mmol = 60 mg)

2,9x60=174mg.

Weigh out 174 mg of urea and add it to 1 litre of stabilised serum, mix gently and thoroughly to dissolve.

10   The total product must now be mixed thoroughly and dispensed into suitable clean, dry, amber-wloured bottles or vials, capped firmly and stored at 4⁰C or at -20⁰C.   If amber-coloured containers are not available, then the serum must be protected from the light to avoid destruction of the bilirubin.

4.3 --   Preparation of a low concentration quality control serum

1   Start with 1 litre of fresh bovine (porcine or equine) serum.

2   Filter through non absorbent cotton wool to remove any large aggregates.

3   Measure the potassium concentration.

4   Calculate the final volume to which 1 litre of serum must be adjusted to dilute the potassium to 3,5 mmol/l. For example, if the measured potassium is 5,4 mmol/l then:

final volume = $\dfrac{5.4}{3,5}$ x 1000 = 1543 ml

(DO NOT ADD WATER YET)

5   15% of 1543 ml is $\dfrac{1543 \times 15}{100}$ = 231 ml

6   Therefore to 1 litre of serum with potassium concentration of 5,4 mmol/l you must add the following:

(a)   Ethylene glycol; 231 ml

(b)   Distilled water; 312 ml (ie, 543 - 231 = 312 ml)

7   Measure the concentration of the analytes in which you are interested and construct a table identical to Table 3, filling in your own measured concentrations (column 4).

8   Adjust the glucose and sodium to the desired concentrations as follows (assuming a final volume for the low concentration serum of 1543 ml);

(a)   Glucose

Amount to be added to 1 litre =

desired concentration - measured concentration;

ie, from the example in Table 3;

3,0 - 2,0 = 1,0 mmol/l

1 mmol = 180 mg.

The total volume of the low concentration serum is 1543 ml (1,543 l), so the amount to be added is 180 x 1,543 = 278 mg. Weigh out 278 mg glucose and add it to the serum.

(b)   Sodium

Amount to be added to 1 litre =

desired concentration - measured. concentration;

ie, from Table 3;

125 - 106 = 19 mmol/l

1 mmol = 58,4 mg of sodium chloride

19 mmol =   x 19 = 1110 mg of sodium chloride.

The total volume of the low concentration serum is 1543 ml (1,543 l) so the amount to be added is 1110x 1,543 = 1713 mg = 1,71 g. Weigh out 1,71 g of sodium chloride and add it to the serum.

Table 3 - APPROXIMATE MEASURED AND DESIRED CONCENTRATIONS OR ACTIVITIES FOR LOW CONCENTRATION QUALITY CONTROL SERUM (BOVINE ORIGIN)

CONSTITUENT	UNITS	DESIRED CONCENTRATION"	MEASURED CONCENTRATION*
Albumin	g/l	30	30
Alk Phos	U/l	40	40
Amylase	U/l	-	-
AST	U/l	60	60
Bicarbonate	mmol/l	11	11
Bilirubin	µmol/l	-	-
Calcium	mmol/l	2,0	2,0
Creatinine	µmol/l	70	70
Glucose	mmol/l	3,0	2,0
Potassium	mmol/l	3,2	3,2
Sodium	mmol/l	125	106
Total protein	g/l	51	51
Urea	mmol/l	3,2	3,2

*   Or activity

9   Mix gently but thoroughly to ensure that the glucose and sodium chloride dissolve completely.

10   The total product must now be mixed thoroughly and dispensed into suitable bottles or vials, capped firmly and stored at 4⁰C or at -20⁰C.

4.4   Preparation of a high concentration quality control serum

1   Start with 1 litre of fresh bovine (porcine or equine) serum.

2   Carefully mix to ensure homogeneity and freeze at -20⁰C. It is important to avoid possible deterioration of this serum by allowing it to remain at ambient temperature too long. The remainder of the process must be completed within one working day.

3   Allow to thaw at room temperature. DO NOT DISTURB OR MIX.

4   When completely thawed remove 300 ml of the top layer of fluid.

5   Add 123 ml of ethylene glycol.

6   Mix very carefully and filter through non absorbent cotton wool to remove any large aggregates.

7   Measure the concentration of the analytes in which you are interested and construct a table identical to Table 4, filling in your own measured concentrations (column 4).

8   Fill in the desired concentrations in column 3 and use that table to calculate the amounts of analytes to be added as described below.

9   The concentrations may be similar to the values given in column 4 of Table 4 with the desired concentrations indicated in column 3. It is anticipated that adjustments will be required for alkaline phosphatase, amylase, bilirubin, creatinine, glucose and urea. The adjustments are based on a final volume of 858 ml (ie, 700 ml concentrated serum, 123 ml ethylene glycol, 30 ml from bilirubin and creatinine adjustments and 5 ml extra ethylene glycol).

Table 4 - APPROXIMATE DESIRED AND MEASURED CONCENTRATIONS OR ACTIVITIES FOR HIGH CONCENTRATION QUALITY CONTROL SERUM (BOVINE ORIGIN)

CONSTITUENT	UNITS	DESIRED CONCENTRATION*	MEASURED CONCENTRATION"
Albumin	g/l	45	45
Alk Phos	U/l	250	60
Amylase	U/l	700	0
AST	U/l	95	95
Bicarbonate	m mol/l	17	17
Bilirubin	µmol/l	150	5
Calcium	m mol/l	3,00	3,00
Creatinine	µmol/l	450	110
Glucose	m mol/l	20	3,0
Potassium	mmol/l	6,0	4,8
Sodium	mmol/l	159	159
Total protein	g/l	76	76
Urea	mmol/l	15	4,8

*   Or activity

10   Adjust the concentration of analytes as follows:

(a)   Alkaline phosphatase
Amount to be added to 1 litre = desired activity - measured activity;
ie, from the example in Table 4;
250 - 60 = 190 units to be added to 1 litre
volume of high concentration serum is 858 ml (0,858 l)
190 x 0,858 = 163 units

163 units of alkaline phosphatase must be added to the high concentration serum; this may be added as a powder or a concentrated suspension. See Appendix 1 for details of the preparation of human placental alkaline phosphatase and Appendix 2 for possible commercial sources of the enzyme.

(b)　Amylase

Amount to be added to 1 litre = desired activity - measured activity;

ie, from the example in Table 4;

700 - 0 = 700 units to be added to 1 litre

volume of high concentration serum is 858 ml (0,858 l)

700 x 0,858 = 601 units

601 units of amylase must be added to the high concentration serum.

Human saliva contains about 1000 units of amyiase/ml, so that 0,6 ml contains 600 units. Collect about 1 ml of saliva, centrifuge at about 2500 rpm for 10 minutes, then add 0,6 ml to the high concentration serum. Mix thoroughly. Measure the amylase activity of the serum at 37°C to confirm that it is approximately 700 U/l.

(c)　Bilirubin

Amount to be added to 1 litre =

desired concentration - measured concentration;

ie, from the example in Table 4;

150 - 5 = 145 μmol/l (1 μmol = 0,59 mg)

145 x 0,59 = 85,6 mg to be added to 1 litre

volume of high concentration serum is 858 ml (0,858 l)

85,6 x 0,858 = 73 mg.

Weigh out 73 mg of bilirubin, dissolve in 15 ml of 0,1 mol/l sodium hydroxide to produce a clear red solution; when the bilirubin has completely dissolved, add the solution, with constant stirring, to the high concentration serum. The procedure should not be carried out in a brightly lit place and, after adding the bilirubin to the control serum, it must be protected from light to avoid destruction of the bilirubin. Mix thoroughly.

(d)　Creatinine

Amount to be added to 1 litre =

desired concentration - measured concentration;

ie, from the example in Table 4;

450 - 110 = 340 μmol/l (1 μmol = 0,113 mg)

340 x 0,113 = 38 mg to be added to 1 litre

volume of high concentration serum is 858 ml (0,858 l)

38 x 0,858 = 33 mg.

Weigh out 33 mg of creatinine, dissolve in 15 ml of 0,1 mol/l hydrochloric acid and when completely dissolved add the solution to the high concentration serum. Mix thoroughly.

(e)   Glucose

Amount to be added to 1 litre =

desired concentration - measured concentration;

ie, from the example in Table 4;

20 - 3 = 17 mmol/l (1 mmol = 180 mg)

17 x 180 = 3,06 g to be added to 1 litre

volume of high concentration serum is 858 ml (0,858 l)

3,06 x 0,858 = 2,63 g.

Weigh out 2,63 g of glucose, add it to the high concentration serum, mix gently to dissolve. Mix thoroughly.

(f)   Urea

Amount to be added to 1 litre =

desired concentration - measured concentration;

ie, from the example in Table 4;

15 - 4,8 = 10,2 mmol/l (1 mmol = 60 mg)

10,2 x 60 = 612 mg to be added to 1 litre

volume of high concentration serum is 858 ml (0,858 l)

612 x 0,858 = 525 mg.

Weigh out 525 mg of urea, add it to the high concentration serum, mix gently to dissolve. Mix thoroughly.

(g)   Final adjustment of ethylene glycol concentration

The final concentration of ethylene glycol must be 15%. It is therefore necessary to add a further 5,0 ml ethylene glycol because the total volume has been increased by 30 ml through the addition of the bilirubin and creatinine solutions. Mix very thoroughly.

11   The total product must now be mixed thoroughly and dispensed into suitable clean, dry, amber-coloured bottles or vials, capped firmly and stored at 4°C or at -20°C. If amber-coloured containers are not available, then the serum must be protected from light to avoid destruction of the bilirubin.

GENERAL NOTES

We recommend that when possible clean and aseptic techniques should be used to reduce the possibilities of contamination.

Prior to analysis the serum should be allowed to rise to ambient temperature, and mixed carefully. It should be kept in the dark to avoid destruction of the bilirubin.

Serum preserved with ethylene glycol may not be suitable for methods employing dialysis.

APPENDIX 1

PREPARATION OF ENZYME CONCENTRATES'

This section gives a detailed description of the preparation of a number of enzyme concentrates from tissue sources which can be used to fortify batches of control serum.

(i)　　　ALKALINE PHOSPHATASE, ALP (EC 3.1.3.1)

ALP is prepared from human placenta using a method based on the extraction procedure of Morton (1950). The placenta is homogenised using 2 ml water per g tissue, then n-butanol is added 1 ml per g tissue and stirred for about one hour. After centrifugation the aqueous layer is fractionated with acetone at 4^0C, the precipitate formed between 33% and 50% (v/v) acetone is retained, dissolved in 0,01 mol/l tris-HCl buffer, pH 7,7 and dialysed overnight against the same buffer. The average yield is 52 units per g tissue and the ALP:AST ratio is greater than 57:1 and ALT activity is undetectable.

This enzyme and all other enzyme preparations described below are distributed in small vials and stored at -20°C until required. The activity of each is measured by the difference before and after addition using a suitable dilution in serum.

(ii)　　　ALANINE AMINOTRANSFERASE, ALT (EC 2.6.2.2)

ALT is prepared from human liver using a procedure based on the method published by Owen et al (1974) for the extraction of ALT from dolphin muscle. Human liver, classified as normal by the pathologist, is homogenised in 0,05 mol/l tris-HCl buffer, pH 7,0 containing 10 mmol/l 2-mercaptoethanol and 10 mmol/l EDTA, 3 ml buffer being used per g of tissue. After centrifugation, the supernatant is placed in a water-bath at 60^0C, brought to 52^0C, then rapidly cooled in an ice-bath. The heat treated sample is fractionated with ammonium sulfate at 4^0C. The precipitate formed when a further 16 g ammonium sulfate per 100 ml are added contains most of the ALT activity and should be separated, dissolved in and dialysed overnight against 0,015 mmol/l tris-HCl buffer, pH 7,25, containing 2 mmol/l EDTA and 10 mmol/l 2-mercaptoethanol. The supernatant from this latter step is retained for AST preparation. The average yield is 14 units per g tissue and the ALT:AST ratio 4,5:1. The ALP activity in this preparation is negligible.

Ox, pig and horse livers do not yield satisfactory ALT preparations, the ratio of AST:ALT activity in the initial extract being very much greater, about 10:1 compared with 2:I in human liver.

(iii)　　　ASPARTATE AMINOTRANSFERASE, AST (EC 2.6.1.1)

AST may also be extracted from human liver by the above procedure. A further 14 g ammonium sulphate per 100 ml are added to the supernatant from the ALT preparation. The precipitate formed contains most of the AST activity. The precipitate is dissolved in and dialysed against 0,015 mol/l tris buffer, to remove ammonium sulfate and is then suitable for addition to quality control material. The average yield is 25 units per g tissue and the AST:ALT ratio 25:1.

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1 Reprinted from Practical Guidelines for the Preparation of Quality Control Sera for Use in Clinical Chemistry by A P Kenny & R H Eaton, WHO document LAB/81.4.

APPENDIX 2

SOME POSSIBLE COMMERCIAL SOURCES OF ENZYMES

ALKALINE   PHOSPHATASE

Sigma Chemical Company

      Catalogue   number   P5521

BDH Chemical s Limited

      Catalogue   number   39035

Merck

      Catalogue   number   1607L

AST

Sigma Chemical Company

      Catalogue   number   G2751

AMYLASE

Sigma Chemical Company

      Catalogue   number   AI 031

BDH Chemicals Limited

      Catalogue   number   39118

Merck

      Catalogue   number   1329

The products satisfactorily used for the evaluation of this procedure were from Sigma Chemical Company listed above.

ACKNOWLEDGEMENTS