

QUALITY ASSURANCE IN HAEMATOLOGY

Prepared on behalf of the World Health Organization

by

Dr S.M. Lewis, B.Sc., M.D., DCP, FRCPath.

Director, WHO Collaborating Centre for Quality Assurance in Haematology  
Chairman, International Committee for Standardization in Haematology

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## PREFACE

For a diagnostic laboratory to serve any real purpose the results of tests from that laboratory must be reliable. Reliability is based on precision and accuracy, with harmonization of results when a test is performed by different methods or instruments either in the same laboratory or in different laboratories. This is especially important when treatment and management of a patient is based on the results of laboratory tests, but reliable haematology data are also necessary for population screening in health surveys and to establish reference values.

Quality assurance is an important component of good laboratory practice and is essential for achieving reliability. This manual provides an introduction to the principles and methods of Quality Assurance in Haematology together with a series of exercises which illustrate these principles based on the common tests which are performed in the haematology laboratory. The exercises will give the trainees an opportunity to become familiar with the recommended techniques, to achieve precision and accuracy in carrying out the tests and to appreciate the causes of error and how to avoid them. The course of exercises is intended for senior laboratory technicians, medical officers with some laboratory experience, directors of haematology laboratories and the staff of the central laboratories and institutes who are involved in the development of national quality assessment programmes. All laboratory staff at senior technician level or above should be able to apply the general principles of internal quality control in their work, interpret quality control charts and carry out tests on material received in an External Quality Assessment (EQA) Scheme. In every laboratory at least one senior technician should be able to prepare various quality control materials. Staff of laboratories directly involved in organizing EQA schemes also need to know how to prepare the quality control materials as well as how to dispatch specimens, process data statistically and report results in such schemes. The directors of central laboratories, responsible for the organization of national quality assurance programmes, should be familiar with all aspects of the subject.

Standardization is a component of quality assurance; this includes standardization of methods and the use of reference standards (i.e. material standards). Over the years there has been close collaboration between the World Health Organization and the International

Committee for Standardization in Haematology for developing standards which appertain to haematology. The role and use of standards in the laboratory is illustrated in the training course exercises which are included in this manual.

The manual is divided into four parts. Part 1 describes the educational objectives; Part 2 deals with the principles and methods of Quality Assurance; Part 3 consists of the training course manual and practical work book, and Part 4 provides detailed instructions for the preparation of calibration and control materials.

This manual is based on earlier documents (LAB/84.3: The Principles and Methods of Quality Assurance in Haematology and LAB/84.6: A Training Course Manual and Practical Work Book). These have been revised in the light of practical experience gained from their use in WHO training courses.

## PART 1: EDUCATIONAL OBJECTIVES

### 1. INTRODUCTION

The text provided in Part 2 of this manual is sufficient for a basic understanding of the principles of quality assurance and the relevant use of simple statistics by all workers who fall into the categories mentioned in the preface.

After having completed a general course based on this document, participants should be familiar with the general principles and practice of internal quality control procedures and should be able to work out the relevant statistics, prepare control charts, interpret the data, make decisions on validity of tests in their laboratories and perform basic tests in haematology for the detection and classification of anaemias.

In addition, some selected participants will also be able to prepare control materials for internal quality control of basic laboratory haematological tests and supervise the use of materials in their laboratories. They should also then be able to organize an External Quality Assessment Scheme, prepare the materials for use in the scheme, analyse results and identify poor performance and resolve problems in tests, materials and instruments used by individual participants in the scheme.

It is expected however, that the course material will also be used in teaching activities where the teacher has enough personal experience to formulate introductory lectures to the various subjects, to stimulate discussions and to reply to questions. From this base further lectures and discussion sessions or workshops may be developed to treat in greater detail the organization of a national quality assurance programme, directed specifically to staff of central laboratories or institutes who may be responsible for the organization of such national programmes.

At least some of the participants in a course should be able to serve as the organizers, lecturers and tutors for courses at a national or local level. To ensure that such courses are comprehensive they should include the discussions and lectures which are listed below. However, the programme and selection of lectures should be based on local laboratory facilities which may vary from country to country.

### 2. PROGRAMME OF DISCUSSIONS AND LECTURES

#### 1. General principles of quality assurance

- 1.1 Objectives
- 1.2 Importance
- 1.3 Terminology used:

- Internal quality control
- External quality assessment
- Quality assurance
- Proficiency surveillance
- Interlaboratory trials
- Quality assurance programme
- Accuracy
- Precision
- Specificity
- Sensitivity
- Reference preparation (material)
- Calibrator
- Control material
- Definitive method
- Reference method
- Reference reagent
- Selected method
- Specimen
- Sample

- 1.4 National, regional and individual laboratory aspects of quality assurance
2. Internal quality control
  - 2.1 Principles of elementary statistics
  - 2.2 Choice and use of calculators
  - 2.3 Specimen collection, preservation, transport and storage
  - 2.4 Specifications of control materials
  - 2.5 Accuracy, precision and bias
  - 2.6 Duplicate tests on patients' specimens
  - 2.7 Check tests
  - 2.8 Tests on control material
  - 2.9 Control charts
  - 2.10 CUSUM chart
  - 2.11 Use of patients' data
3. External quality assessment
  - 3.1 Organization of external quality assessment schemes (EQAS)
  - 3.2 Preparation of material
  - 3.3 Analysis of quantitative data
  - 3.4 Deviation index
  - 3.5 Youden plot and identification of bias
  - 3.6 Analysis of qualitative results
  - 3.7 Identification of poor performance
  - 3.8 Inter-relationship of national and international EQAS
  - 3.9 WHO programme
4. Uses of EQAS
  - 4.1 State of art
  - 4.2 Evaluation of kits, reagents, instruments, controls and calibrators
  - 4.3 Interlaboratory comparability
  - 4.4 Establishing "truth" by consensus, reference laboratories and definitive methods
  - 4.5 EQAS as base for training programmes and laboratory improvement
5. Identification of causes of problems in individual laboratories
  - 5.1 Technique
  - 5.2 Samples
  - 5.3 Equipment
  - 5.4 Methods
  - 5.5 Environment
  - 5.6 Staffing
  - 5.7 Working space
  - 5.8 Limits of acceptable performance
  - 5.9 Clinical usefulness
  - 5.10 Limits of accuracy
6. Elucidation of causes of error
  - 6.1 Specimen collection
  - 6.2 Specimen tube and anticoagulant
  - 6.3 Transit time and environment
  - 6.4 Specimen mix-up
  - 6.5 Unrepresentative sampling
  - 6.6 Faulty pipettes and pipetting errors
  - 6.7 Instrument faults
  - 6.8 Incorrect instrument setting/filter
  - 6.9 Inadequate use of reference and control preparation
  - 6.10 Lack of quality control system
  - 6.11 Errors of calculation

- 6.12 Reporting and recording errors
- 6.13 Inaccurate or inappropriate dilution
- 6.14 Incorrect method (e.g. wrong diluent, inadequate reaction)
- 6.15 Wrong interpretation of observation

7. Standardization

- 7.1 Specifications for reference standards
- 7.2 Preparation of reference standards
  - 7.2.1 Characteristics of reference preparations used in different test principles (cell counting, chemical analysis, coagulation, microscopy)
- 7.3 Preparation of control materials
- 7.4 Preparation of calibrators
- 7.5 Definitive, reference and selected methods
- 7.6 Establishment of national reference values
- 7.7 Relationship between national and international standardization
- 7.8 How an international standard is developed: Role of WHO and ICSH
- 7.9 Calibration charts and tables

8. Production of materials for internal quality control and external quality control

- 8.1 At national level
- 8.2 By individual laboratories

9. Qualitative quality control

- 9.1 Blood films
- 9.2 Bone marrow preparations
- 9.3 Special procedures including cytochemistry

10. Assessment by correlation

- 10.1 Cumulative reports and charts
- 10.2 Correlation between various tests
- 10.3 Blood films
- 10.4 Clinical status

11. Test and equipment selection

- 11.1 Evaluation of kits
- 11.2 Evaluation of a test method
- 11.3 Evaluation of instruments and check of manufacturer's claims
- 11.4 Selection of appropriate tests and instruments
- 11.5 Check of colorimeters, cell-counters and other apparatus in use
- 11.6 Calibration of pipettes

12. The role of the laboratory director in quality assurance

- 12.1 Inspection of quality control data
- 12.2 Cost of quality control
- 12.3 Efficiency and effectiveness in specimen processing and reporting
- 12.4 Training programme for staff
- 12.5 Education of the user of the laboratory
- 12.6 Self audit
- 12.7 Laboratory safety with regard to choice of technique and handling of material and equipment

13. Principles of reference values

- 13.1 Frequency histograms and determination of population distribution
- 13.2 "Normal" range, "usual" range and "reference" range
- 13.3 Direct measurements and derived values

PART 2: PRINCIPLES AND METHODS OF QUALITY ASSURANCE

1. INTRODUCTION

It is the responsibility of the person in charge of the haematology laboratory, whether he or she be doctor or technician, to ensure that the tests which are performed are relevant and that the results are reliable, reproducible and as accurate as possible according to the present state of the art. The results must be presented, without undue delay, to the clinician or public health worker who has requested the tests, in a report which is legible and readily understood. The aim is to ensure that they serve their intended purpose, whether it be to provide help for a clinical diagnosis and for management of a patient, or for health care of a population at large.

It is obvious that the person in charge of the laboratory must ensure that the tests are performed as soon as possible after the specimens have reached the laboratory, with technical skill, and with appropriate controls and reference materials to be assured of their technical reliability. But it must be remembered that he must also be aware of variables which are not always under his immediate control but which might significantly influence the test results. These include the effects on the blood count and on other blood components of exercise and mental stress just before blood is collected, the position of the patient (standing, sitting, lying in bed), a tourniquet left on the arm for too long before venepuncture, collection of capillary blood as compared to venous blood. The blood count, coagulation tests and certain chemical tests will be affected by incorrect anticoagulant, or by excess or deficient concentration of anticoagulant, by inadequate mixing of the specimens with the anticoagulant, by delays in sending the specimens to the laboratory and leaving them in an unfavourable environment such as room temperature in a hot climate, and, even worse, in direct sunlight. It is also important to ensure that the specimen containers do not leak, not only because contamination of the laboratory (and ward) staff is a potential health hazard, but also because a container without a secure cap is likely to disturb the constituents and their relationship, by evaporation of plasma and by leakage. The laboratory director must maintain close contact with the wards and also with the suppliers to ensure that these many factors are taken into account before a specimen actually arrives at the laboratory.

To achieve the necessary level of good performance and to be reassured that this level is constantly maintained, it is necessary to undertake a programme of quality assurance.

There are three separate aspects of such a programme, namely, internal quality control, external quality assessment and surveillance of proficiency.

Internal quality control is intended to monitor various aspects of test procedures which are performed in the laboratory. It includes measurement on specially prepared materials, repeated measurements on routine specimens as well as statistical analysis, day by day, of data obtained from the routine tests which have been carried out in the laboratory. These measures provide a way to achieve precision (i.e. reproducibility) but not necessarily also accuracy.

External quality assessment is the objective evaluation by an outside agency of performance by a number of laboratories on material which is supplied specially on a regional or national basis for this purpose. The objective is to achieve comparability and possibly also accuracy if the material supplied for the tests has been assayed by a reference laboratory, using methods of known precision, alongside a reference preparation of known value.

Surveillance proficiency implies critical supervision of all aspects taking account of general organization of the laboratory service, including specimen collection, labelling, delivery and storage before the tests are performed, efficiency of recording and reporting of results, maintenance and control of equipment and apparatus, staff training, protection of laboratory staff against health risks and hazards when handling specimens and equipment. This requires an understanding of the principles of good management as well as technical knowledge of the test procedures and an appreciation of the likely causes of inaccuracy and

Imprecision in the test results. Technical aspects are dealt with in laboratory manuals<sup>1</sup> and will not be described here. The purpose of this document is to introduce the principles and practice of internal quality control for the individual laboratory worker and to provide guidelines for participation in an external quality assessment scheme. Staff working in rural or district hospital laboratories may also be expected to organize and supervise a limited scheme for external quality assessment of any tests performed at health centres. Similarly, the staff of regional laboratories might be expected to provide a service for rural hospital laboratories. Accordingly, instructions for providing an external quality assessment scheme on a limited scale are included.

Laboratories vary in the way in which they are organized, the facilities which are available, the range of tests which they are able to undertake and their workload; they also vary in the number of staff and in their levels of training. It is, thus, not possible to give a rigid protocol for a quality assurance programme with procedures which must be carried out, nor even to indicate which procedures are more important than others. Each laboratory should establish a programme, as determined by local circumstances, with procedures selected from the list on page 21.

## 2. CONTROL MATERIALS

In Part 4 (page 48) technical details are given for the production of material which is suitable for use in quality control of blood counts. As the blood count constitutes the main work of most "routine" haematology laboratories, it is of considerable importance to have these preparations available as they can be used for a number of different purposes. Human or equine (i.e. donkey or horse) blood is used for some of the procedures for checking precision in internal quality control. The same material is used for external quality assessment and also as a calibrator.

When used as a control to check the precision of a test, it is not necessary to know the true concentration of the substance to be measured. But when the material is intended for use as a calibrator, it must have an assigned value; to obtain this value the test should be carried out by a reference method, and checked with an international reference preparation if one has been established. The measurements are made only after the preparation has been dispensed as aliquots in small (e.g. 2-5 ml) volumes in vials. The measurement must be made on at least 10 vials taken at random from the batch, and the results recorded as mean ( $\bar{x}$ ) and standard deviation (SD).

Intrabatch variation (expressed as CV%, i.e.  $\frac{SD}{\bar{x}} \times 100$ ) should be within the limits stated in the methods for measurement described below.

As measurement of haemoglobin (Hb) and blood cell counting (especially total leucocyte count [WBC]) are the most common tests in routine haematology, it is in this area that quality control is especially required, and will be described in this document. However, the principles also apply to other tests including quantitative tests on whole blood, e.g. Hb A<sub>2</sub>; and tests on plasma or serum (e.g. iron, iron binding capacity, vitamin B12, folate). The descriptions given in a later section can be easily adapted for these tests. Obviously, this approach does not apply to qualitative tests such as identification of abnormal haemoglobins, blood film morphology, etc; methods of quality control for these types of tests are described in the books referred to above. The blood count procedures apply equally to manual methods and the use of cell counters.

<sup>1</sup> Evatt, B.L., Lewis, S.M., Lothe, F & McArthur, J.R. Fundamental Diagnostic Laboratory Haematology: The Anaemias. CDC, Atlanta, and WHO, Geneva, 1984.

Dacie, J.V. & Lewis, S.M. Practical Haematology, 7th ed. Churchill Livingstone, Edinburgh, 1989.

Lewis, S.M. & Verwilghen, R.L. Quality assurance in haematology. Ballière Tindall, London, 1988.

### 3. STATISTICS OF QUALITY CONTROL

There are four features used in the procedures which will be described in the sections which follow. These are:

- Mean ( $\bar{x}$ )
- Standard deviation (SD)
- Coefficient of variation (CV)
- Median (m)

#### 3.1 Mean ( $\bar{x}$ )

This is simply the total score of all the measurements divided by the number of measurements. Thus, for example, if the haemoglobin is measured on a specimen 11 times, with the following results:

126 120 118 124 115 110 112 115 122 120 115 (g/l)

then

$$126 + 120 + 118 + 124 + 115 + 110 + 112 + 115 + 122 + 120 + 115 = 1297,$$
$$m = 1297 \div 11 = 117.909$$

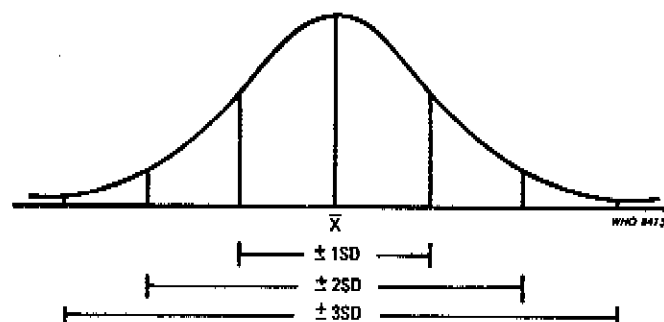
For subsequent statistical calculations retain one figure after the decimal point, e.g. 117.9.

For practical purposes, round up (or down). In this example, record as 118 g/l.

#### 3.2 Standard deviation (SD)

All measurements obtained in laboratory tests show variation. To understand the significance of SD in the context of quality control, the way in which the test results are likely to lie above and below the mean must be appreciated. When the results all belong to the same "populations" (set) their distribution will be symmetrical and this can be expressed as a Gaussian curve. This curve has the following appearance:

FIG. 1 NORMAL DISTRIBUTION CURVE (GAUSSIAN CURVE)



The measure of spread is conveniently expressed by the SD. The area under the centre of the curve is  $\pm 1SD$  and this equals 68% of the whole area;  $\pm 2SD$  equals 95%;  $\pm 3SD$  equals 99.7%.

Calculation of the SD is useful in quality control procedures. If the limits are set at  $\pm 2SD$ , when 100 measurements are performed, say of haemoglobin, then 95% of the 100 will fall within  $\pm 2SD$ . This also means that by chance alone 5 out of 100 determinations will be outside the 95% range.

3.3 Calculation of standard deviation

SD is calculated from the formula:  $SD = \sqrt{\frac{\text{sum of } d^2}{n - 1}}$

where "d" is the difference of each individual result from the mean, and "n" is the number of measurements. It may also be expressed as:

$$\sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}} \text{ where "x" = individual measurement and "\bar{x}" = mean.}$$

This calculation is readily obtainable by using a calculator which has this function, by means of which the SD may be obtained directly merely by entering the test results. It can, however, also be obtained by direct calculation. The method for this is shown in the following example with Hb:

- Carry out a number of consecutive measurements of Hb on a specimen. The usual number of measurements may be 10-20
- Tabulate the measurements in a column (headed "x"). Calculate mean (=  $\bar{x}$ ).
- For each x, calculate  $x - \bar{x}$  and enter in a second column.
- Then calculate  $(x - \bar{x})$  ( $x - \bar{x}$ ), i.e.  $(x - \bar{x})^2$  and enter in a third column.
- Calculate  $\sqrt{\frac{\sum(x - \bar{x})^2}{(n - 1)}}$ . In the example given below this equals

$$15.36 = 3.9192, \text{ i.e. } 3.9.$$

Thus, SD = 3.9, or practically 4 g/l.

Test No.	x	(x - $\bar{x}$ )	(x - $\bar{x}$ ) <sup>2</sup>
1	155	+ 4	16
2	148	- 3	9
3	152	+ 1	1
4	147	- 4	16
5	150	- 1	1
6	156	+ 5	25
7	156	+ 5	25
8	157	+ 6	36
9	153	+ 2	4
10	150	- 1	1
11	150	- 1	1
12	147	- 4	16
13	144	- 7	49
14	152	+ 1	1
15	157	+ 6	36
16	152	+ 1	1
17	147	- 4	16
18	152	+ 1	1
19	145	- 6	36
20	150	- 1	1
<hr/>			
Totals	20	3020	292

$$\text{Thus } \bar{x} = \frac{3020}{20} = 151 \quad \frac{\sum(x - \bar{x})^2}{n - 1} = \frac{292}{19} = 15.36$$

3.4 Coefficient of variation (CV)

This relates the SD to the actual measurement so that measurements at different levels can be compared:

CV (as a percentage) is calculated by  $\frac{SD \times 100}{\text{mean}}$ .

Thus, using the example given above,  $CV = \frac{3.9}{151} \times 100 = 2.58\%$ .

In another situation, for example, if the Hb = 60 g/l and SD = 3.9, then  $CV = \frac{3.9}{60} \times 100 = 6.5\%$ ; thus, at this level, the test is being performed with a much lower precision. When the Hb is 60 g/l, to obtain a CV of 2.5%, the SD must not exceed 1.5 g/l.

### 3.5 Median (m)

The median is the point on the scale that has an equal number of observations above and below. It is especially useful as an alternative to mean when there are extreme values so that the mean does not give a true picture.

It is determined as the mid-point value, i.e. there are as many measurements above as below.

When the number of measurements (n) is even, media will be midway between  $(\frac{n}{2})$  and  $(\frac{n}{2} + 1)$ .

When n is an odd number m will be at the position of  $\frac{n+1}{2}$ .

#### Example

Using the measurements provided in 3.1 on page 9, tabulation will set them out as follows:

	<u>g/l</u>
1.	110
2.	112
3.	115
4.	115
5.	115
6.	118
7.	120
8.	120
9.	122
10.	124
11.	126

$$n = 11$$

$$\frac{n+1}{2} = 6\text{th position.}$$

$$\therefore \text{Median (m)} = 118 \text{ g/l}$$

### 3.6 Standard error of mean (SEM)

Although the mean is usually calculated and presented as a single measurement, there will be some dispersion around that figure. The extent of this variation is indicated by the SEM which is the SD of the mean. This is calculated by  $\frac{SD}{\sqrt{n}}$ . Thus, using the above data  $SEM = \frac{3.9}{\sqrt{20}} = 0.87$  (i.e. 0.9).

## 4. INTERNAL QUALITY CONTROL (IQC)

4.1 Duplicate tests on patients' specimens

This is the easiest procedure to carry out; if the day's work allows it, every specimen should be tested in duplicate; if there are too many test requests for this to be practical, test at least 10 consecutive specimens in duplicate from time to time.

Calculate the standard deviation:  $SD = \sqrt{\frac{\text{sum of } d^2}{2n}}$

where  $d^2$  = difference between duplicates squared,  
n = number of specimens tested in duplicate.

Interpretation

None of the duplicate tests should differ from each other by more than 2SD as calculated. This is a method to identify random errors. If the test is always badly done the SD will be wide and will not be sensitive to individual errors.

ExampleWBC ( $\times 10^9/l$ )

<u>Specimen</u>	<u>1st count</u>	<u>2nd count</u>	<u>d</u>	<u>d<sup>2</sup></u>
1	5.4	5.8	0.4	0.16
2	8.3	10.5	2.2	4.84
3	17.2	18.0	0.8	0.64
4	5.4	5.4	0	0
5	12.2	11.8	0.4	0.16
6	14.3	13.8	0.5	0.25
7	6.2	6.4	0.2	0.04
8	8.2	8.6	0.4	0.16
9	7.3	7.5	0.2	0.04
10	5.4	5.9	0.5	0.25

$$\Sigma d^2 = 6.54$$

$$\frac{\Sigma d^2}{2n} = \frac{6.54}{20} = 0.327$$

$$\sqrt{\frac{\Sigma d^2}{2n}} = 0.5718$$

Therefore SD = 0.57 2SD = 1.14

Conclusion

Test on specimen 2 is unsatisfactory and must be repeated.

4.2 Check tests

These are similar to duplicate tests but they use specimens which have been measured originally in an earlier batch.

Interpretation

The tests should agree with each other within  $\pm 2SD$ . This procedure will detect deterioration of apparatus and reagents which may have developed between tests if it is certain that there has been no deterioration in the specimens on storage. Thus, this test is suitable for Hb, less so for RBC, WBC and it is unsuitable for PCV if there is a delay of six hours or longer between the two tests.

It is useful to use the same specimens for check tests and duplicate tests. The SD can be established on the basis of technical competence from duplicate tests; if the SD for the check tests is greater this is a clear indication of deteriorating apparatus or reagents PROVIDED THAT THE SPECIMENS HAVE NOT ALTERED.

#### 4.3 Replicate tests on control specimens

Repeated measurements on a single specimen will define the error of reproducibility (precision) and it is a method for evaluating technical excellence and/or an instrument which is unstable. As all the measurements are carried out with the same pipettes and reagents, it will not detect faults in these.

This test can be performed on any suitable blood sample but it is useful to use control material. When the SD of the repeat measurements has been obtained, as described below, this can be used for the control chart which is described in the next section. For Hb, use the lysate or preserved blood.

For replicate testing do 11 identical tests on one sample.

Calculate mean.

Calculate the difference from mean for each measurement.

Calculate  $SD = \sqrt{\frac{\sum d^2}{n - 1}}$  where  $\sum d^2$  = sum of squared differences.

Calculate  $CV = \frac{SD \times 100}{\text{mean}} \%$ .

#### Interpretation

The SD and CV give an index of precision. The desirable level of precision should be such that errors caused by the measurement procedure do not significantly affect clinical interpretation of the measurement. Thus, for example, if a clinician usually diagnoses that a haemorrhage has occurred when the haemoglobin falls by 10% of its previous level, it becomes necessary to be confident that the CV of the test is less than 5%. This means that the SD should not be greater than 4 g in 80 g/l or 8 g in 160 g/l. The desirable level of precision should be such that errors induced by the measurement process do not affect the range of values for the normal population. This objective is achieved if the SD is less than 1/12 of the defined "normal range" in a healthy population (i.e. the limits into which 95% of a normal population fall). Thus, in the case of haemoglobin, the "normal range" for men is usually given as 130-170 g/l. The test SD should thus be less than 4 g/l; the CV is then approximately 2.5-3%.

With some tests, such as RBC by haemocytometry, it is not possible to achieve the desirable level of precision by the usual technique in the routine laboratory, as the CV is usually in the order of 10%. On the other hand, with automated cell counters one expects a CV of 2% for the RBC, as these instruments have a remarkably high level of precision.

#### Example

Consecutive measurements of Hb (g/l):

142 141 146 144 143 140 146 150 150 143 146

Mean ( $\bar{x}$ ) =  $\frac{1591}{11}$  = 144.6

Set out in columns:

No.	Measurement	$d = (x - \bar{x})$	$d^2 = (x - \bar{x})^2$
1	142	2.6	6.76
2	141	3.6	12.96
3	146	1.4	1.96
4	144	0.6	0.36
5	143	1.6	2.56
6	140	4.6	21.16
7	146	1.4	1.96
8	150	5.4	29.16
9	150	5.4	29.16
10	143	1.6	2.56
11	146	1.4	1.96

$$\Sigma d^2 = 110.56$$

$$\frac{\Sigma d^2}{n - 1} = 11.05$$

$$\sqrt{\frac{\Sigma d^2}{n - 1}} = \sqrt{11.05} = 3.32$$

$$SD = 3.3$$

$$CV = \frac{3.32 \times 100}{144.6} = 2.30, \text{ i.e. } 2.3\%$$

#### Conclusion

Although there was a 10 g/l difference between highest and lowest levels, overall haemoglobin measurement is being performed with an acceptable level of precision for clinical purposes.

#### 4.4 Control chart

This uses the mean and SD obtained on the control material (lysate or preserved blood) as its basis.

Using arithmetic graph paper, calibrate the vertical scale in appropriate units, e.g. Hb in g/l and the horizontal scale in days or batches of tests. Draw a horizontal line to represent the mean Hb. Draw two other lines above and below the mean, respectively, to represent + 2SD and -2SD. In the example given, the following figures were obtained:

Mean: 144.6 g/l      SD = 3.3 g      2SD = 6.6 g

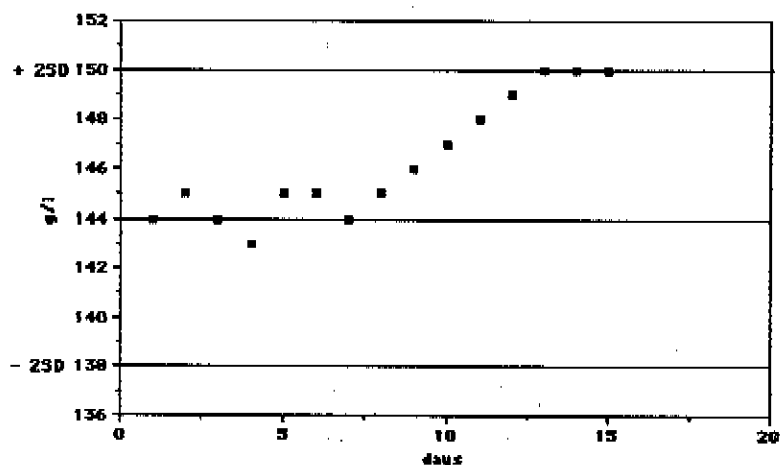
In rounding the figures it is convenient to plot the mean as:

144 g/l;      +2SD as 150 g/l;      and -2SD as 138 g/l.

With each batch of routine specimens include a sample of the control material and plot the result on the graph paper. This will give at least one value per working day. In the example shown, over a 15-day period the control material gave the following sequential results:

144 - 145 - 144 - 143 - 145 - 145 - 144 - 145 - 146 - 147 - 148 - 149 - 150 - 150 - 150 g/l

FIG. 2



### Interpretation

When the test is in control, all the measurements on successive samples will approximate to the established mean, with only minor deviations which will oscillate above and below the line of the mean. The chart will suggest that there is a fault in technique, instrument, pipette or reagents if one of the following occurs:

- (1) a value entirely outside the  $\pm 2SD$  control limits;
- (2) several consecutive values showing a rising or falling tendency;
- (3) several consecutive values on one side of the mean;
- (4) two or more results per 20 on the  $+2SD$  or  $-2SD$  lines.

But first check that the material itself has not become infected or in other ways has begun to deteriorate.

An example of good control and an incident where a new reagent resulted in faulty performance is shown in the control chart illustrated in Fig. 2. The arrow indicates where the fact that the test was out of control should have been recognized and steps taken to identify and correct the fault.

### Cumulative sum (CUSUM) control

This is another way to display the data obtained in the test for precision. The CUSUM is the running total of the differences between successive measurements and the mean which was established initially. The plus and minus signs must be taken into account.

This is thought to be a more sensitive indicator of faulty technique or equipment. It is especially useful for detecting a consistent change in performance due to drift, as there will be a progressing increase in the deviation (plus or minus). By contrast, when there are only random differences from the mean some will be positive and some will be negative, i.e. plus and minus, so that the CUSUM will oscillate around zero. The CUSUM can be presented either as a chart on arithmetic graph paper or as a Table.

#### (a) CUSUM chart

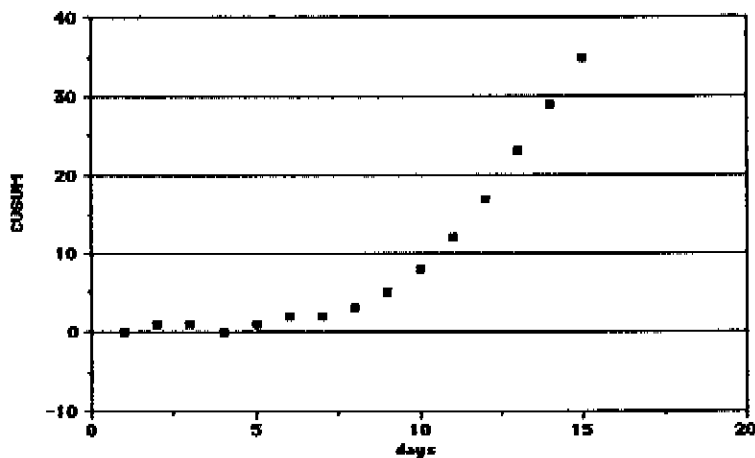
Using arithmetic graph paper calibrate the vertical scale in appropriate units, e.g. Hb in g/l. Calibrate the horizontal scale in units (e.g. one day or one batch of routine specimens). A narrow scale will emphasize the gradient and this will demonstrate a change in slope and allow the detection of significant trends readily.

An example of the use of a CUSUM chart is shown in Fig. 3. This has been drawn using the same data as for the control chart (Fig. 2 above).

Mean Hb on control material = 144 g/l.

Day	Hb g/l	CUSUM
1	144	0
2	145	+ 1
3	144	+ 1
4	143	0
5	145	+ 1
6	145	+ 2
7	144	+ 2
8	145	+ 3
9	146	+ 5
10	147	+ 8
11	148	+ 12
12	149	+ 17
13	150	+ 23
14	150	+ 29
15	150	+ 35

FIG. 3



Inspection of this chart should have alerted the laboratory earlier than by using the control chart. The vertical scale refers to deviation from the established mean (Hb in g/l).

(b) CUSUM table

1. Decide on minimum significant change to be detected  
=  $d$  (usually  $2SD$ )
2. Calculate " $k$ " =  $d/2$
3. Calculate upper reference value (URV)  
= mean +  $k$   
lower reference value (LRV)  
= mean -  $k$

4. Calculate the Decision interval = 2.0 k
5. Method of CUSUM result recording:
  1. Set CUSUM at zero.
  2. Do not start the CUSUM if the control value lies between URV and LRV.
  3. Start CUSUM if control value (x) lies outside URV or LRV. The CUSUM figure is the difference between the control specimen value and the appropriate RV i.e. either  $x-URV$  or  $LRV-x$
  4. To this difference add the next CUSUM difference and continue to add sequentially the difference between the control value and the same reference value, even when the control value falls within the reference values or outside the opposite reference value.
  5. If the CUSUM sign changes but the value is not outside the opposite reference value, set to zero and start a new CUSUM.
  6. If the CUSUM sign changes but the value is outside the opposite reference value, this indicates a possible abrupt shift in calibration.
  7. If the CUSUM equals or exceeds the decision interval this suggests a significant change in accuracy: check calibration, correct as necessary, set CUSUM to zero and start a new CUSUM.

Using the above data, the following results are obtained:

$$2SD = 6.6 \text{ g/l} \quad \text{Mean} = 144.6 \text{ g/l}$$

$$k \left( \frac{d}{2} \right) = 3.3 \text{ g/l}$$

$$URV = 147.9 \quad LRV = 141.3$$
$$\text{Decision Interval} = 6.6$$

On days 1-10 results were within the URV and LRV limits, thus requiring no action.  
On day 11 result was 0.1 above URV; CUSUM was thus 0.1  
On day 12 result was 1.1 above URV; CUSUM was thus  $0.1 + 1.1$   
On day 13 result was 2.1 above URV; CUSUM was thus  $1.2 + 2.1 = 3.3$   
On day 14 result was 2.1 above URV; CUSUM was thus  $3.3 + 2.1 = 5.4$   
On day 15 result was 2.1 above URV; CUSUM was thus  $5.4 + 2.1 = 7.5$   
At this stage CUSUM was greater than the decision interval: test out of control.

#### 4.5 Patients' data

In a large hospital where at least 100 blood counts are performed each day, there should be no significant day-to-day or week-to-week variability in the means of red cell indices (MCV, MCH, MCHC). This is one method for quality control in laboratories with automated cell counters, as any significant change may indicate a change in instrument calibration or a fault in its function provided that the specimens are evenly distributed (see below). The more sophisticated automated cell counting systems have a computer programme incorporated in the system which makes it possible to analyse the data continuously. With other counters results can also be analysed using a desk-top programmable calculator. By this means results are analysed in successive batches of 20 patients' specimens. Readers who require further information on the way in which this procedure can be used with an automated system should refer to one of the text books listed in the footnote on page 8.

Results are valid only if the population from whom the routine specimens are received does not vary significantly from day to day and the tests are not selectively biased. Such a bias might occur if, for example, as a result of specific outpatient clinics, tests are carried out on only certain days of the week on patients with iron deficiency or with some other condition which affects the MCHC, MCH and/or MCV.

A simple adaptation of the same principle can be applied in laboratories using manual methods and which do not have computer facilities. In this situation the procedure is confined to the MCHC. The mean MCHC is calculated at the end of each day on all the measurements obtained during the course of the day. If the test is being performed satisfactorily, the mean will not vary by more than 2SD on any day. Before setting up this procedure it is necessary to establish the SD. This is done by calculating the mean on 11 consecutive working days and then calculating the SD.

$$SD = \frac{\sum(x - \bar{x})^2}{n - 1}$$

where  $x$  = daily mean,  $\bar{x}$  = mean of daily means and  
 $n$  = number of days, so that  $n - 1 = 10$ .

It is convenient and educative to plot the data on a graph which should be updated each day. This method applies primarily to the control of absolute values. The component indices (Hb, RBC count and PCV) are controlled only indirectly by this procedure.

4.6 Correlation check

This implies that any unexpected result of a test must be checked to see whether it can be explained on clinical grounds or whether it correlates with other tests. Thus, for example, an unexpectedly higher or lower haemoglobin might be explained by a blood transfusion or a haemorrhage, respectively. A low MCHC should be confirmed by demonstrating hypochromic red cells on a Romanowsky-stained blood film; similarly the blood film should be examined to confirm a leucocytosis or leucopenia, a thrombocytosis or thrombocytopenia - but be careful as the blood film itself may be misleading if not correctly made and stained.

Recording blood count data on cumulative report forms is good clinical practice as well as providing an inbuilt quality control system by making it easy to detect an aberrant result when compared with a previously determined baseline. This is especially useful in detecting the occasional wild errors caused by incorrect labelling, inadequate mixing or partial clotting of a blood sample. An example of a record card is illustrated in Fig. 4.

FIG. 4

NAME		Sex/Age	Ward	Case No.	Consultant	Diagnosis													
SMITH, John		M/48		56144	Dr. A. B.	AI Haemolytic Anaemia													
Date	R.B.C. 10 <sup>12</sup> /L	Hb. g/l	P.C.V.	M.C.H.C.	M.C.V.	M.C.H.	Reticulocytes %	W.B.C. 10 <sup>9</sup> /L	Blasts	Promyelocytes	Myeloblasts	Metamyelocytes (Young forms)	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Platelets 10 <sup>9</sup> /L	Other Data
14/3																			
15/3	3.28	113	34	33	105	35	6.5	5.1					64	0	1	24	11	133	
5/4	3.28	111	34	32	104	34	7.2	7.5										136	
12/4	2.27	75	22	33	98	33	6.7	5.2										140	← Haemolytic episode Haemoglobinuria
13/4	2.14	72	21	35	95	33	14.2	5.0				3	48	0	2	28	19	155	
16/4	1.97	67	18	35	94	34	18.0	7.3										165	
17/4	3.17	103	29	34	92	32	2.2	6.0					50	0	1	41	8	115	← Transfusion: 4 units blood
20/4	3.27	102	30	33	91	31	1.4	5.1										134	

## 5. EXTERNAL QUALITY ASSESSMENT

Even when all possible precautions are taken to achieve reliable results by internal quality control, errors will arise which are detectable only by external assessment; moreover, this is an essential process for ensuring harmonization or "transferability" of results between laboratories, and to recognize systematic errors. In haematology there are few tests which can be performed with such certainty by a definitive method that the result can be guaranteed as being accurate in the sense of absolute truth. Harmonization enables all laboratories to achieve results which are in agreement with each other and which will thus be clinically meaningful; external quality assessment identifies any laboratory which does not achieve this. The principle is that samples of the same material are sent from one centre (national or regional) to a large number of laboratories where the requested tests are performed. The materials described in Part 4 are suitable for this purpose. All the laboratories send their results back to the centre where they are analysed and interpreted in one of the three ways described below.

### 5.1 Consensus method

From the results the centre calculates a mean and SD. The SD is calculated in the usual way by the formula:

$$SD = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

where  $x$  = individual results,  
 $\bar{x}$  = mean  
 $n$  = total number of results

The data are then adjusted by excluding any which are  $>2SD$  from the mean. The mean and SD (now termed "weighted") are then recalculated. A "deviation index" (DI) is then calculated for each individual participant. This indicates the difference between the individual laboratory results and the weighted mean and can be used to compare the performance of a laboratory with that of other laboratories as well as with its own performance in previous surveys.

The formula for calculating DI is:

$\frac{\text{Actual result} - \text{weighted mean for test}}{\text{Weighted SD}}$ , eliminating the minus sign if the actual result is smaller than the weighted means for the test.

When there is a non-Gaussian distribution (see page 9) it is sometimes better to use median ( $m$ ) instead of mean ( $\bar{x}$ ) in calculating deviation index. Thus  $DI = \frac{x - m}{\text{weighted SD}}$ . See page 10 for method of obtaining measurement of  $m$ .

The deviation index score for any test may be interpreted as follows:

<0.5	= excellent performance
0.5-1.0	= satisfactory performance
1.0-2.0	= still acceptable
>2.0	= defect requiring attention

For this scheme to be valid, there must be at least 20 participants in a survey, and at least half the participants should have sufficiently good performance in comparison with each other to avoid having a (weighted) SD which is unhelpfully wide.

### 5.2 Assigned value method

As described in Part 4, when calibration and control materials are made it is possible to assign values by using reliable methods, as far as possible internationally recommended methods together with international reference preparations. It is also possible to establish the reproducibility for each test as performed by one or more skilled technicians in the national or regional centre. Thus, results from participants can be judged by the extent of the deviation from the assigned mean.

- <1SD = excellent performance
- 1-2SD = satisfactory performance
- 2-3SD = defect needing attention
- >3SD = serious problem needing urgent attention

This scheme is valid no matter how few participants there are. Its limitation is that there is no assurance that the methods used in assigning values are themselves free of bias. It is advisable for the centre to use several different techniques for each test, and preferably for the tests to be performed in three different laboratories which have been designated as reference centres because of their expertise.

### 5.3 Linked scheme

The World Health Organization sponsors a project in which selected laboratories in different countries take part in an international scheme. This is organized by the WHO Collaborating Centre for Quality Assessment in Haematology in association with the United Kingdom national scheme (NEQAS). Selected material used for surveys in the latter scheme is also sent to the international participants who later receive a report containing the results of the United Kingdom survey. As the statistical evaluation in the United Kingdom NEQAS is based on over 700 results which are obtained by various techniques, including several different types of automated cell counters, the consensus is unlikely to be affected by bias, whilst random errors are taken into account by the statistical treatment of the data. Because the majority of results are obtained with automated counters which have a high degree of precision, after excluding a small number of outliers with "wild" results the SD and CV are remarkably low. Some of the specimens used for certain tests which are included in the surveys are measured by reference methods, and limits for satisfactory performance are set as described on page 13.

Typical results in a United Kingdom NEQAS trial are given in Fig. 5.

FIG. 5

	m**	All methods*		n
		SD	CV	
Hb g/l	122	1.5	1.2	724
RCC x 10 <sup>12</sup> /l	4.04	0.08	2.0	618
PCV	0.372	0.01	3.6	680
MCV fl	93	2.82	3.0	607
MCH pg	30.3	0.67	2.2	592
MCHC g/l	32.7	11.1	3.4	609
WCC x 10 <sup>9</sup> /l	13.0	0.67	5.1	714

\* Results are also analyzed separately for different counting systems and methods.

\*\* m = Median (see page 11, section 3.5)

From the mean or median and SD of the United Kingdom NEQAS surveys the international participants can judge their performance critically, although their own results will not have been included in the statistical evaluations. The intention is that the laboratories taking part in the WHO scheme may subsequently be responsible for organizing regional or national schemes which will thus be linked at an international level. Further information is obtainable from the Director of the WHO Collaborating Centre.<sup>1</sup>

### 6. QUALITY ASSURANCE PROGRAMME

Every laboratory must undertake a programme of quality assurance. Every type of test undertaken by the laboratory must be subjected to some form of quality assurance, whether by

<sup>1</sup> Dr S.M. Lewis, Royal Postgraduate Medical School, Ducane Road, London W12 0NN, United Kingdom.

way of internal quality control, external quality assessment or both. The procedures which should be included in a programme in any individual laboratory will vary with which tests are undertaken, the equipment (especially if this includes a fully automated counting system), the size of the laboratory and level of training of its staff and the number of specimens handled each day. At least some of the following programme must be carried out.

**AT ALL TIMES**

**Correlation system:**

Cumulative report forms  
Correlation of blood film appearances with blood count

**DAILY**

**Tests on control specimens:**

Control sample with each batch of specimens  
Control chart  
CUSUM chart  
Duplicate measurements on a few of the patients' specimens - usually 3-5 in each batch.  
Check test on a few patients' specimens from a previous batch (usually 3-5).

**Statistical analysis:**

If by automated counters - means of MCV, MCH, MCHC  
If by manual methods - mean of MCHC

**AT INTERVALS (DAILY OR WEEKLY)**

**Calibration of counters, spectrophotometers and other instruments:**

Haemoglobin - haemiglobincyanide reference preparation  
- lysate  
  
RBC - preserved blood  
- stabilized red cell preparation  
  
WBC - stabilized avian blood  
Platelets - stabilized human platelet preparation

**AT INTERVALS (MONTHLY OR THREE-MONTHLY)**

National or regional EQA scheme

**AT INTERVALS (INITIALLY AND AGAIN WHEN INDICATED)**

Calibration of pipettes and automatic dispensers.

**6.1 Method for calibration of micropipettes**

The barrel of a tuberculin syringe is lubricated and the tip of the syringe is then attached by means of a piece of thick rubber or plastic tubing to the base of the pipette to be calibrated. The unit is clamped vertically on a retort stand. The plunger of the syringe is withdrawn slightly and the pipette is submerged in a beaker containing mercury at room temperature held beneath the unit. The pipette is then filled with mercury by further aspiration with the syringe. When the mercury has reached the calibration mark of the pipette the beaker is removed. A weighing bottle, the weight of which has been determined, is held beneath the pipette and the measured amount of mercury delivered into it by manipulation of the syringe. The weight of the bottle plus the mercury is determined and the weight of the mercury alone is then obtained by subtraction. The ambient temperature is noted.

The volume of the pipette (in  $\mu\text{l}$ ) is calculated by dividing the weight of mercury (in mg) by one of the following factors depending on temperature:

<u>Temperature (<math>^{\circ}\text{C}</math>)</u>	<u>Factor</u>
18-21	13.55
22-25	13.54
26-28	13.53
29+	13.52

The calibration must be performed in duplicate for each pipette.

#### 7. CONTROL OF PERFORMANCE OF PHOTOMETER

To ensure that a photometer is functioning correctly a calibration graph should be prepared when it is first put into use in the laboratory, and thereafter at intervals - usually every six months, but every one to two weeks if there is any doubt about its performance. The following example illustrates the preparation of a calibration graph for use in haemoglobinometry, but the same principles also apply to other tests.

In preparing a calibration graph, a series of five cuvettes or tubes is set up. Into these tubes the following amounts of haemiglobincyanide (HiCN) reference preparation are pipetted by using a 10 ml graduated pipette.

- Tube 1: 6 ml, approximately
- Tube 2: 4.5 ml, accurately measured
- Tube 3: 3.0 ml, accurately measured
- Tube 4: 1.5 ml, accurately measured
- Tube 5: none

After the reference solution has been pipetted, the pipette is rinsed through with haemiglobincyanide reagent. The rinsings are discarded and, with the same pipette, reagent is added to the five tubes as follows:

- Tube 1: none
- Tube 2: 1.5 ml, accurately measured
- Tube 3: 3.0 ml, accurately measured
- Tube 4: 4.5 ml, accurately measured
- Tube 5: 6 ml, approximately

The contents of each tube are well mixed.

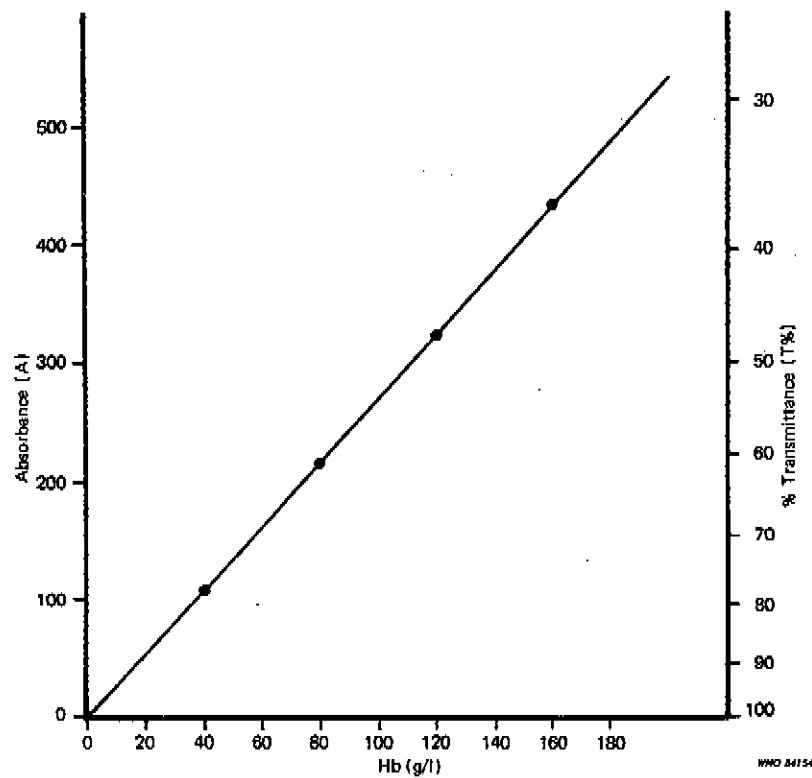
The haemoglobin concentration in the five tubes will be, respectively: labelled value (full strength);  $3/4$  times labelled value;  $1/2$  times labelled value;  $1/4$  times labelled value; zero strength (blank).

With a filter at 540 nm, the cuvette containing reagent only (tube 5) is placed into the holder, and the instrument is set at zero absorbance (100% transmittance). The contents of tubes 1, 2, 3 and 4 are transferred to clean, dry, matched cuvettes.

The outsides of the cuvettes are wiped carefully free of moisture, fingerprints, or lint. The cuvettes are placed successively into the instrument, and the readings are taken.

If the readings are in absorbance (A), they are plotted on the vertical scale and haemoglobin concentration on the horizontal scale of metric graph paper. If readings are in percentage transmittance, semilogarithmic paper must be used, with the transmittance plotted on the vertical (log) scale. The points should fall on a line passing through zero (see Fig. 6).

FIG. 6



In practice, the concentration of haemoglobin in blood which is diluted appropriately can be read from the calibration graph or, preferably, from a table which can be constructed from the values which were obtained. If the line of the calibration graph is not linear throughout its length, only the linear portion can be used for deriving measurements. If there is no linearity at all, the instrument requires attention by the manufacturer.

PART 3: PRACTICAL EXERCISES FOR TRAINING COURSE

INTRODUCTION

In the exercises which follow, haemoglobinometry and other fundamental haematological tests have been used as the model by means of which the principles and practical application of quality assurance are demonstrated. This section should be used in conjunction with the CDC-WHO manual "Anemia - Fundamental Diagnostic Hematology". A by-product of the course is that the participants will have received training in techniques which are used for the detection and classification of anaemia; furthermore, they will have been introduced to the use of standards and methods for standardization.

It is recommended that the practical exercises be performed by both the individual laboratory workers and organizers of External Quality Assessment Schemes. In this they should consider their different roles. Thus in the exercises every participant should be regarded as an "individual laboratory" and his or her result should be analysed individually in terms of Internal Quality Control; at the same time all results should be analysed communally for External Quality Assessment. When the principles have been mastered with fundamental haematological tests the practice of quality assurance can be extended into other tests, especially those which have a particular importance in diagnosis or health screening surveys in the area where the participants work.

Exercise 1: Calculation of standard deviation, variance and coefficient of variation.

The following data were obtained in 10 consecutive haemoglobin measurements on two colorimeters A and B.

<u>Test</u>	<u>A</u> <u>Hb g/l</u>	<u>B</u> <u>Hb g/l</u>
1	155	150
2	148	135
3	152	145
4	147	137
5	150	153
6	156	163
7	157	155
8	153	149
9	150	144
10	150	137

Calculate for each series of readings:

- (a) Mean ( $\bar{x}$ )
- (b)  $\Sigma(x - \bar{x})^2$
- (c) Variance ( $s^2$ )

$$= \frac{\Sigma(x - \bar{x})^2}{n - 1}$$

- (d) Standard deviation (SD)

$$= \sqrt{s^2}$$

- (e) Coefficient of variation (CV) which is a percentage =  $\frac{SD}{\bar{x}} \times 100$

- (f) Standard error of mean (SEM) =  $\frac{SD}{\sqrt{n}}$

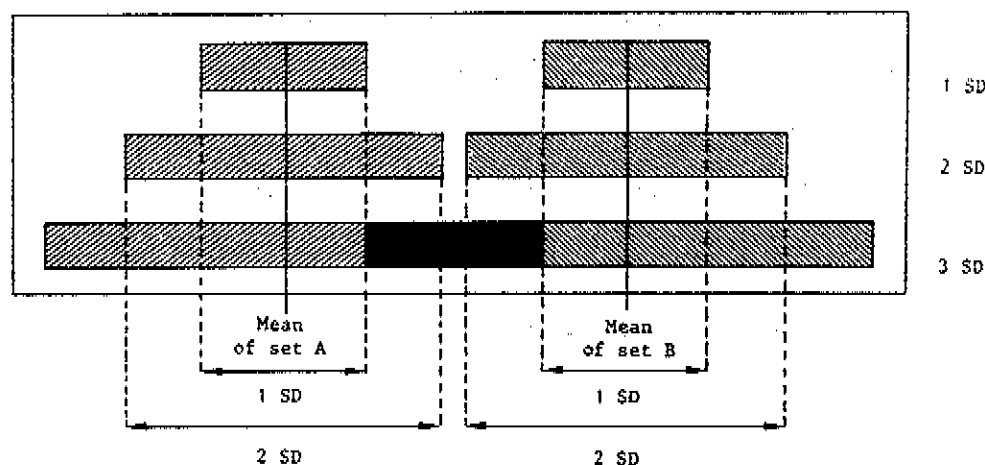
where  $x$  represents the individual data;  $\bar{x}$  is the mean; and  $n$  the number of data items in the sets.

**Exercise 2: Differences between means**

The simplest method for assessing whether two sets of data are similar to or different from each other is to calculate their means and SDs. If the ranges, i.e. means  $\pm$  SDs, do not overlap, the sets are significantly different. Thus, for example if the mean and SD are  $40 \pm 3$  and  $50 \pm 3$  respectively, their 1 SD range would be 37-43 and 47-53; clearly separate sets. Conversely, if their values were  $40 \pm 10$  and  $50 \pm 10$  their 1 SD range would be 30-50 and 40-60: with this overlapping the sets cannot be regarded as being separate entities.

If there are differences between the two sets, the extent of difference depends on whether they can be separated at 3 SD when there is a 99% probability, or at 2 SD when there is a 95% probability, or at 1 SD when there is only a 66% probability, that the difference is really significant. In other words when the sets appear separate at 1 SD range, this does not necessarily mean that they will remain separated at the 3 SD range, as illustrated in Fig. 7.

FIG. 7



This method assumes that the mean is a constant figure, and it does not take account of dispersion around that figure. This dispersion is indicated by standard error of mean (see page 10).

A more reliable method is to analyse the standard error of the difference between the means (SE diff). In this procedure the difference between two sets of data is regarded as significant only if their means differ from each other by an amount greater than the SE diff. This is obtained by the following calculation:

Standard error of difference in means (SE diff) =

$$\sqrt{\frac{(SD_1)^2}{n_1} + \frac{(SD_2)^2}{n_2}}$$

where  $SD_1$  and  $SD_2$  are the SDs of set 1 and 2 respectively,  
 $n_1$  and  $n_2$  are the number of data items in the sets.

Using both the above methods (i.e. SD of means and SE diff) determine whether there is significant difference between the measurements by the two colorimeters in Exercise 1.

This will indicate the extent of a systematic discrepancy (bias). Variations due to random factors which are not constant can be demonstrated by analysis of variance, as described in Exercise 3.

Exercise 3: Analysis of variance

There are several ways in which the SDs of two sets of data can be compared statistically to determine whether there are significant differences between them. One convenient way is with the "F-ratio", which compares the variances ( $s^2$ ) of the two sets.

$$F\text{-ratio} = \frac{s^2 \text{ of set A}}{s^2 \text{ of set B}}$$

The ratio must not be less than 1; accordingly, select the set with the greater variance as the numerator (i.e. Set A). To determine the significance of the F-ratio as calculated, compare the figure obtained with the figures in the Table at the appropriate degrees of freedom for the two sets of data (degree of freedom = number of measurements - 1). To be significant the calculated ratio must be greater than the figure in the Table, using the lower or the upper part of the Table for 99% or 95% probability, respectively.

Using the data in Exercise 1, calculate the F-ratio of the two sets A and B.

Is there a significant difference in the reliability of the two colorimeters?

Analysis of differences by t-test

This is another method for comparing two sets of data, using one of the following calculations:

(a) Difference in means

$$t = \frac{\bar{x} \text{ of Set A} - \bar{x} \text{ of Set B}}{SE \text{ diff}}$$

(b) Difference in paired results

Difference between pairs = d  
Sum of differences =  $\Sigma d$

$$\text{Mean of differences} = \frac{\Sigma d}{n} = \bar{d}$$

$$\text{Variance } (s^2) = \frac{\Sigma (d - \bar{d})^2}{n - 1}$$

$$SE \text{ mean of differences} = \sqrt{\frac{s^2}{n}}$$

$$t = d \div \sqrt{\frac{s^2}{n}}$$

Interpretation: From chart, find t-distribution for appropriate degree of freedom (n - 1). Express results as level of probability (p) that there is no significant difference between means or sets of pairs, e.g. with df = 19:

t = 0	1.25	1.65	5.65	$\infty$
p = 100%	20-30%	10-20%	.1-.05%	0%
i.e. p = 1	.3>p>.2	.2>p>.1	.001>p>.0005	p = 0

As a rough guide, when  $t > 4$  there is a significant difference between the two instruments both for means and for paired results.

F Distribution, Upper 5% Points (F .95)

		Degrees of Freedom for Numerator																		
Degrees of Freedom for Denominator	$F_{.95}$	1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	$\infty$
	1	161.4	199.8	216.7	224.6	230.9	234.0	236.8	238.9	240.5	241.9	243.9	245.9	248.0	249.1	250.1	251.1	252.2	253.3	254.3
2	18.51	19.00	19.18	19.26	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45	19.45	19.46	19.46	19.47	19.48	19.49	19.50
3	10.13	9.88	9.82	9.82	9.81	9.81	9.80	9.80	9.80	9.79	9.79	9.79	9.79	9.79	9.79	9.79	9.79	9.79	9.79	9.79
4	7.71	6.94	6.80	6.79	6.78	6.78	6.78	6.78	6.78	6.78	6.78	6.78	6.78	6.78	6.78	6.78	6.78	6.78	6.78	6.78
5	6.61	5.79	5.61	5.60	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59
6	5.99	5.14	4.95	4.94	4.93	4.93	4.93	4.93	4.93	4.93	4.93	4.93	4.93	4.93	4.93	4.93	4.93	4.93	4.93	4.93
7	5.58	4.74	4.53	4.52	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.51
8	5.32	4.48	4.27	4.26	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25
9	5.12	4.28	4.06	4.05	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.04
10	4.96	4.10	3.87	3.86	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85
11	4.84	3.98	3.75	3.74	3.73	3.73	3.73	3.73	3.73	3.73	3.73	3.73	3.73	3.73	3.73	3.73	3.73	3.73	3.73	3.73
12	4.76	3.89	3.66	3.65	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64
13	4.67	3.81	3.58	3.57	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56
14	4.60	3.74	3.51	3.50	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49	3.49
15	4.54	3.68	3.45	3.44	3.43	3.43	3.43	3.43	3.43	3.43	3.43	3.43	3.43	3.43	3.43	3.43	3.43	3.43	3.43	3.43
16	4.49	3.63	3.40	3.39	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38
17	4.45	3.60	3.37	3.36	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35
18	4.41	3.55	3.32	3.31	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30
19	4.38	3.52	3.29	3.28	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27
20	4.35	3.48	3.25	3.24	3.23	3.23	3.23	3.23	3.23	3.23	3.23	3.23	3.23	3.23	3.23	3.23	3.23	3.23	3.23	3.23
21	4.32	3.47	3.24	3.23	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22
22	4.30	3.44	3.21	3.20	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19
23	4.28	3.42	3.19	3.18	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17
24	4.26	3.40	3.17	3.16	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15
25	4.24	3.39	3.16	3.15	3.14	3.14	3.14	3.14	3.14	3.14	3.14	3.14	3.14	3.14	3.14	3.14	3.14	3.14	3.14	3.14
26	4.23	3.37	3.15	3.14	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13
27	4.21	3.35	3.14	3.13	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12
28	4.20	3.34	3.13	3.12	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11
29	4.18	3.33	3.13	3.12	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11
30	4.17	3.32	3.12	3.11	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10
40	4.08	3.23	3.04	3.03	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02
60	4.00	3.15	2.96	2.95	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94
120	3.92	3.07	2.88	2.87	2.86	2.86	2.86	2.86	2.86	2.86	2.86	2.86	2.86	2.86	2.86	2.86	2.86	2.86	2.86	2.86
$\infty$	3.84	3.00	2.80	2.79	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78

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F Distribution, Upper 1% Points (F .99)

		Degrees of Freedom for Numerator																			
		1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	$\infty$	
Degrees of Freedom for Denominator	$F_{.99}$	4088	4999-6	6408	6925	7784	8888	9928	9981	9922	9086	8106	8167	8209	8255	8281	8287	8313	8329	8366	
	$F_{.99}$	1	98.50	99.00	99.17	99.25	99.30	99.33	99.36	99.37	99.39	99.40	99.42	99.43	99.45	99.46	99.47	99.47	99.48	99.49	99.50
2	84.18	80.82	79.46	78.71	78.24	77.91	77.67	77.49	77.36	77.23	77.05	76.87	76.69	76.59	76.50	76.41	76.32	76.22	76.13	76.04	
3	81.20	78.00	76.69	76.08	75.63	75.31	75.08	74.90	74.76	74.63	74.45	74.27	74.09	73.99	73.90	73.81	73.72	73.62	73.53	73.44	
4	79.28	76.13	74.85	74.28	73.85	73.54	73.32	73.14	73.00	72.87	72.69	72.51	72.33	72.23	72.14	72.05	71.96	71.86	71.77	71.68	
5	77.99	74.88	73.63	73.08	72.67	72.37	72.15	71.97	71.83	71.70	71.52	71.34	71.16	71.06	70.97	70.88	70.79	70.69	70.60	70.51	
6	77.07	74.00	72.77	72.23	71.83	71.53	71.31	71.13	71.00	70.87	70.69	70.51	70.33	70.23	70.14	70.05	69.96	69.86	69.77	69.68	
7	76.42	73.38	72.17	71.64	71.25	70.95	70.73	70.55	70.42	70.29	70.11	69.93	69.75	69.65	69.56	69.47	69.38	69.28	69.19	69.10	
8	75.94	72.93	71.73	71.20	70.81	70.51	70.29	70.11	69.98	69.85	69.67	69.49	69.31	69.21	69.12	69.03	68.94	68.84	68.75	68.66	
9	75.56	72.57	71.38	70.85	70.46	70.16	69.94	69.76	69.63	69.50	69.32	69.14	68.96	68.86	68.77	68.68	68.59	68.49	68.40	68.31	
10	75.24	72.27	71.09	70.56	70.17	69.87	69.65	69.47	69.34	69.21	69.03	68.85	68.67	68.57	68.48	68.39	68.30	68.20	68.11	68.02	
12	74.78	71.83	70.66	70.13	69.74	69.44	69.22	69.04	68.91	68.78	68.60	68.42	68.24	68.14	68.05	67.96	67.87	67.77	67.68	67.59	
15	74.32	71.39	70.23	69.70	69.31	69.01	68.79	68.61	68.48	68.35	68.17	67.99	67.81	67.71	67.62	67.53	67.44	67.34	67.25	67.16	
20	73.86	70.95	69.80	69.27	68.88	68.58	68.36	68.18	68.05	67.92	67.74	67.56	67.38	67.28	67.19	67.10	67.01	66.91	66.82	66.73	
24	73.50	70.60	69.46	68.93	68.54	68.24	68.02	67.84	67.71	67.58	67.40	67.22	67.04	66.94	66.85	66.76	66.67	66.57	66.48	66.39	
30	73.20	70.31	69.18	68.65	68.26	67.96	67.74	67.56	67.43	67.30	67.12	66.94	66.76	66.66	66.57	66.48	66.39	66.29	66.20	66.11	
40	72.94	70.06	68.94	68.41	68.02	67.72	67.50	67.32	67.19	67.06	66.88	66.70	66.52	66.42	66.33	66.24	66.15	66.05	65.96	65.87	
60	72.68	69.81	68.70	68.17	67.78	67.48	67.26	67.08	66.95	66.82	66.64	66.46	66.28	66.18	66.09	66.00	65.91	65.81	65.72	65.63	
120	72.42	69.56	68.46	67.93	67.54	67.24	67.02	66.84	66.71	66.58	66.40	66.22	66.04	65.94	65.85	65.76	65.67	65.57	65.48	65.39	
$\infty$	72.16	69.31	68.21	67.68	67.29	66.99	66.77	66.59	66.46	66.33	66.15	65.97	65.79	65.69	65.60	65.51	65.42	65.32	65.23	65.14	

DF	Probability					
	0.5	0.1	0.05	0.02	0.01	0.001
1	1.000	6.314	12.706	31.821	63.657	636.619
2	0.816	2.920	4.303	6.965	9.925	31.598
3	0.765	2.353	3.182	4.541	5.841	12.941
4	0.741	2.132	2.776	3.747	4.604	8.610
5	0.727	2.015	2.571	3.365	4.032	6.859
6	0.718	1.943	2.447	3.143	3.707	5.959
7	0.711	1.895	2.365	2.998	3.499	5.405
8	0.706	1.860	2.306	2.896	3.355	5.041
9	0.703	1.833	2.262	2.821	3.250	4.781
10	0.700	1.812	2.228	2.764	3.169	4.587
11	0.697	1.796	2.201	2.718	3.106	4.437
12	0.695	1.782	2.179	2.681	3.055	4.318
13	0.694	1.771	2.160	2.650	3.012	4.221
14	0.692	1.761	2.145	2.624	2.977	4.140
15	0.691	1.753	2.131	2.602	2.947	4.073
16	0.690	1.746	2.120	2.583	2.921	4.015
17	0.689	1.740	2.110	2.567	2.898	3.965
18	0.688	1.734	2.101	2.552	2.878	3.922
19	0.688	1.729	2.093	2.539	2.861	3.883
20	0.687	1.725	2.086	2.528	2.845	3.850
21	0.686	1.721	2.080	2.518	2.831	3.819
22	0.686	1.717	2.074	2.508	2.819	3.792
23	0.685	1.714	2.069	2.500	2.807	3.767
24	0.685	1.711	2.064	2.492	2.797	3.745
25	0.684	1.708	2.060	2.485	2.787	3.725
26	0.684	1.706	2.056	2.479	2.779	3.707
27	0.684	1.703	2.052	2.473	2.771	3.690
28	0.683	1.701	2.048	2.467	2.763	3.674
29	0.683	1.699	2.045	2.462	2.756	3.659
30	0.683	1.697	2.042	2.457	2.750	3.646
40	0.681	1.684	2.021	2.423	2.704	3.551
60	0.679	1.671	2.000	2.390	2.660	3.460
120	0.677	1.658	1.980	2.358	2.617	3.373
∞	0.674	1.645	1.960	2.326	2.576	3.291

Exercise 4: Evaluation of new method

Haemoglobin was measured on a set of specimens (A) with the ICSH reference method and (B) with a new method using undiluted blood. Evaluate the new method by t-test on paired results and by demonstrating the extent of correlation or lack thereof by plotting the data on arithmetic graph paper.

	(A) Reference method (g/l)	(B) New method (g/l)	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
1)	70	65			
2)	76	75			
3)	110	120			
4)	90	75			
5)	116	110			
6)	113	105			
7)	97	80			
8)	95	105			
9)	85	65			
10)	64	50			
11)	120	115			
12)	117	115			
13)	101	100			
14)	98	90			
15)	73	60			
			$\Sigma d$		$\Sigma$
			d		

$$s^2 = \frac{\Sigma(d - \bar{d})^2}{n - 1}$$

$$SE \text{ diff} = \sqrt{\frac{s^2}{n}}$$

$$t = d \div SE \text{ diff} = \boxed{\phantom{00}}$$

Exercise 5:

In a trial of a new method for serum iron the results given below were obtained using five control sera whose iron concentration has been determined with a reference method. Results are given in  $\mu\text{g/dl}$ .

Calculate standard deviation, and CV (%) at the five levels. Plot the means of the results obtained with the new method against the reference results on arithmetic graph paper. Comment on the suitability of using the new method as the selected method in your laboratory.

Control serum	Reference method	New method
A	40	51 - 58 - 67 - 61 - 63 - 59 60 - 58 - 61 - 64 - 59 - 59
B	100	126 - 118 - 226 - 121 - 123 - 117 114 - 120 - 122 - 125 - 118 - 120
C	160	184 - 180 - 177 - 185 - 181 - 174 179 - 178 - 180 - 182 - 183 - 177
D	220	244 - 242 - 237 - 240 - 236 - 239 244 - 238 - 240 - 241 - 235 - 244
E	280	278 - 272 - 264 - 270 - 266 - 274 264 - 268 - 270 - 272 - 274 - 268

	A	B	C	D	E
n					
$\bar{x}$					
SD					
CV%					

**Exercise 6: Precision of pipetting**

Fill one 20  $\mu$ l pipette from the sample provided and dilute the measured volume in 4 ml of cyanide-ferricyanide reagent.

Repeat 10 times. Read each in a photoelectric colorimeter at a wavelength of 540 nm or with an appropriate filter (e.g. Ilford 625). Using a haemiglobincyanide reference preparation convert results to Hb (g/l). Keep the diluted samples.

Test No.	Galvo reading converted to Hb g/l (x)	(x - $\bar{x}$ )	(x - $\bar{x}$ ) <sup>2</sup>
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Calculate mean of series ( $= \bar{x}$ )

$$\text{Calculate standard deviation (SD)} = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

$$\text{Calculate CV (\%)} = \frac{SD}{\bar{x}} \times 100$$

$$\text{Calculate SEM} = \sqrt{\frac{SD}{n}}$$

**Exercise 7: Assessment of linearity and reproducibility**

Prepare the material for this exercise as follows:

- (a) From one donor collect approximately 20 ml of venous blood in EDTA. Mix well and transfer approximately 8 ml, 8 ml and 4 ml into three centrifuge tubes, labelled 1, 2 and 3, respectively.
- (b) Centrifuge tubes 1 and 2; take 2 ml of plasma from each and add to tube 3. Mix the contents of tube 3 and relabel as A.
- (c) Mix the contents of tubes 1 and 2, pool together and relabel as E.
- (d) Make 5 samples as follows:
  - Tube A - as above
  - Tube B - 2 ml of A + 1 ml of E
  - Tube C - 2 ml of A + 2 ml of E
  - Tube D - 1 ml of A + 2 ml of E
  - Tube E - as above

Carry out the exercise as follows:

- (a) Using a 20  $\mu$ l pipette make a 1:201 dilution (in duplicate) of each of the samples A-E in cyanide-ferricyanide reagent. Read the optical density (OD) of each in a photoelectric colorimeter at 540 nm (or using an appropriate yellow-green filter).

By means of a haemiglobincyanide reference preparation convert the readings into Hb concentration (g/l).

- (b) Measure the microhaemtocrit of each sample in duplicate.

Record the results on the chart below:

	Fresh samples			After 24 hour storage		
	OD	Hb g/l	PCV	OD	Hb g/l	PCV
A	1					
	2					
B	1					
	2					
C	1					
	2					
D	1					
	2					
E	1					
	2					
HiCN						
Ref						
Prep						

- (c) On arithmetic graph paper mark an arbitrary scale of 100-400 units on the horizontal axis. On the vertical axis mark one scale for OD readings and another one for PCV.

Plot all the measurements obtained with samples A-E - plot A at 100, B at 200, C at 250, D at 300 and E at 400. Draw a line of best fit for the OD measurements and another line for the PCV measurements. Note the following:

- (1) Is any part of the line non-linear?
- (2) Do any of the duplicate measurements not fall close to the line?
- (3) To what extent does the PCV parallel the OD readings?
- (4) Convert the OD readings to Hb concentrations and calculate the SD of the difference between duplicate measurements by the

$$\text{formula } SD = \sqrt{\frac{\sum d^2}{2n}}, \text{ where } d = \text{the differences between paired}$$

measurements, and n = number of paired readings.

Have there been any significant errors in any of the duplicate measurements? This will be the case when the difference in the duplicate measurements is greater than the SD.

- (5) Identify the reliable range of OD in the colorimeter, and express this in terms of Hb concentration at the dilution used.
- (6) Measure the Hb and PCV on the same samples A-E after storage for 24 hours. Record the results. Has there been any significant change in any of the measurements on any of the samples?

Exercise 8: Measurement of haemoglobin

Carry out haemoglobin estimation by the haemiglobincyanide method on whole blood sample A and B; lysed samples C and D; and diluted sample E (as HiCN).

A reference standard will be provided and the results of measurements on a reference spectrophotometer will be given.

	HiCN	HiCN spectrophotometric*
Reference Preparation		
Hb value g/l		
Absorbance (A <sup>540</sup> )		
Sample A		
Absorbance (A <sup>540</sup> )		
Hb (g/l)		
Sample B		
Absorbance (A <sup>540</sup> )		
Hb (g/l)		
Sample C		
Absorbance (A <sup>540</sup> )		
Hb (g/l)		
Sample D		
Absorbance (A <sup>540</sup> )		
Hb (g/l)		
Sample E		
Absorbance (A <sup>540</sup> )		
Hb (g/l)		

\* From formula: 
$$\text{Hb (g/l)} = \frac{A^{540} \times 64500 \times \text{dilution}}{44.0 \times 1000 \times d}$$
 where d = layer thickness of solution in cm (usually 1.000 cm).

Using all the class results, calculate  $\bar{x}$ ,  $s^2$ , SD and CV for each specimen.

HiCN				
	$\bar{x}$	$s^2$	SD	CV
Reference preparation				
Sample A				
B				
C				
D				
E				

Calculate F ratios of various sets. Compare the following and note whether there are significant differences in reliability of measurement of Hb:

1. High concentration (A) versus low concentration (B)
2. High concentration (C) versus low concentration (D)
3. Whole blood (A) versus lysate (C)
4. Whole blood (B) versus lysate (D)
5. Prediluted (E) versus self-diluted. You will be told which of the samples has been used to provide sample E.

**Exercise 9: Haemoglobinometry**

Repeat Exercise 8 on the same samples A, B, C and D using another method, e.g. oxyhaemoglobin or whole blood haemoglobinometer. Using all class results, calculate  $\bar{x}$ ,  $s^2$ , SD and CV for each:

	$\bar{x}$	$s^2$	SD	CV
Reference preparation				
Sample A				
B				
C				
D				

Calculate F ratios and determine whether there is a significant difference in reliability in comparison with HiCN method:

1. Sample A
2. Sample B
3. Sample C
4. Sample D

**Exercise 10:** Preparation of calibration graph/table for haemoglobinometry.

A calibration graph should be prepared whenever a new photometer is put into use in the laboratory and again every six months. By the method described in Part 2, page 20, prepare a graph which relates Hb to absorbance (or transmittance). Check the validity of the graph for reading haemoglobin values. Select an appropriate dilution to be used when measuring haemoglobin on blood samples.

For preparing the graph, use the ICSH HiCN reagent and read the Hb concentration from the graph. Express the results in g/l.

	Absorbance/transmittance	Hb (g/l)
Sample A		
Sample B		

**Exercise 11:** Control chart method for quality control

First make a series of at least 10 measurements of the control material provided and calculate the mean and standard deviation (SD) of the results. Prepare a control chart as described in Part 2, page 13, calibrating the vertical scale in appropriate units (e.g. Hb g/l), and indicate the levels of +2SD and -2SD.

For the exercise the results obtained from a series of measurements of the control material are given below. Plot these on the chart and comment.

The control was a set of samples of preserved blood from one donor. Initially, ten replicate measurements of haemoglobin were obtained from one of the samples (Column A). Then haemoglobin was measured on a different sample each day. Results over 20 days are shown in Columns B and C. All measurements are expressed in g/l.

A	Day	B	Day	C
142	1	142	11	145
141	2	144	12	148
146	3	143	13	148
144	4	143	14	149
143	5	141	15	151
145	6	143	16	151
140	7	145	17	152
143	8	143	18	154
142	9	144	19	154
144	10	142	20	154

Use of cumulative sum method (CUSUM)

The CUSUM technique is described on page 15. Replot the data obtained for the control chart (above) as a CUSUM chart, and check test control by CUSUM calculation.

Exercise 12: Statistical analysis of absolute values means

This is the method of quality control based on the principle that the mean values of the blood count (and especially the absolute values) remain constant in a selected population. You are provided with the MCHC measurements obtained over several successive days from the routine blood counts.

Determine the mean and SD of the first day's data as a base line. Then determine the mean and SD on each subsequent day. Has there been a change in the mean (= systematic error) or an increase in the SD (= random error)?

Repeat the exercise using only data from the blood counts in which the Hb was more than 130 g/l.

Exercise 13: Normal range and reference values

Results given below are the measurements of haemoglobin (in 5 g/l intervals) obtained from a group of apparently normal people in a population. Plot the data as a frequency histogram on arithmetical graph paper and by eye determine the best fit curve. Is the distribution "normal" (i.e. Gaussian)? What is the mode and what is the median?

Calculate mean ( $\bar{x}$ ) and SD.

Set a range of  $\bar{x} \pm 2SD$ .

Eliminate from the original data any values which are outside  $\pm 2SD$ .

Recalculate  $\bar{x}$  and SD of the remaining values. Calculate the range which should include 95% of a normal population (i.e. 2SD). Does this differ from the original group?

Hb (g/l)	No. of times
100	1
105	0
110	1
115	1
120	2
125	1
130	3
135	3
140	7
145	10
150	3
155	4
160	2
165	2
170	0

Exercise 14:

Serum bilirubin was determined in a group of men students, aged 20-24 years. The results (in mg/dl) are given below. Present the figures as a histogram and comment on the distribution. Identify the mode and the median.

Assuming Gaussian distribution calculate the means, and the ranges of  $\pm 2SD$ .

Comment on the validity of this method for the data provided.

0.5	0.5	0.4	0.6
0.4	0.4	0.5	0.5
0.6	0.8	0.7	0.9
0.4	0.5	1.4	0.5
0.3	0.5	1.0	0.6
0.7	0.4	0.6	0.5
0.4	2.2	1.3	0.4
1.1	0.5	2.9	0.4
0.5	0.4	0.4	0.5
0.3	0.3	0.5	0.6
0.9	0.5	0.4	1.0
0.4	0.6	1.5	1.6

Exercise 15: Red cell counts

Carry out a red cell count by counting chamber and electronic counter on the provided blood in EDTA (A) and preserved blood in ACD (B), using appropriate dilution.

Establish mean, count variance ( $\sigma$ ), SD and CV.

	Specimen	Dilution	No. of squares counted	No. of cells counted ( $\lambda$ )	Red-cell count ( $\times 10^{12}/l$ )	$\sigma$ of cell counted ( $\sqrt{\lambda}$ )	Range $\lambda \pm 2\sigma$	Count CV (%)
Counting-chamber counts	A							
	B							
Total class	A							
	B							

$\sigma$  is the theoretical variation between consecutive counts. It conforms to a Poisson distribution and is given as  $\sqrt{\lambda}$ , where  $\lambda$  is the total number of cells counted in the defined volume. It must not be confused with the standard deviation (SD) which is a measure of the variation which actually occurs between results when the test is repeated consecutively.

RBC ( $\times 10^{12}/l$ )		A	B
By class count	$\lambda$		
	$\sigma$		
	CV		
By participant counts	$\bar{x}$		
	SD		
	CV		
By electronic counter			

Note that "Class count" = total number of cells counted by entire class (=  $\lambda$ ).  
 "Participant count" = each member of class provides a result which is used to calculate  $\bar{x}$  and SD. The count variance ( $\sigma$ ) is  $\sqrt{\lambda}$ .

**Exercise 16: Total leucocyte counts.**

By counting chamber and electronic methods:

- (a) Estimate the total leucocyte count in diluted (1:20) blood A provided.
- (b) Estimate the total leucocyte count in blood samples B and C, making suitable dilutions of the blood. B is a fresh blood; C is a control preparation.

	Blood dilutions	No. of $1 \text{ mm}^2$ areas counted	No. of cells counted ( $\lambda$ )	Calculated leucocyte count ( $\times 10^9/l$ )	Count variance $\sigma$ ( $\sqrt{\lambda}$ )	Count CV (%)
Self counts	A 1:20					
	B					
	C					
Total class counts	A					
	B					
	C					
Electronic counts	A					
	B					
	C					

WBC ( $\times 10^9/l$ )	A	B	C
By class count	$\lambda$		
	$\sigma$		
	CV		
By participant counts	$\bar{x}$		
	SD		
	CV		
By electronic counter	$\bar{X}$		
	SD		
	CV		

**Exercise 17: Differential counts**

Stain film provided with a Romanowsky stain, and carry out a differential count.

Total WBC ( $\times 10^9/l$ ) =

Total No. cells counted	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
200					
As %					
$\sigma (\sqrt{\lambda})$					
Total					
2 $\sigma$ Range					
Total class results					
As %					
$\sigma (\sqrt{\lambda})$					
Total					
2 $\sigma$ Range					

**Exercise 18: Platelet count**

Carry out platelet counts on fresh blood sample (A) and control preparation (B) by haemocytometer method, using ammonium oxalate diluent. Examine under phase contrast, if this is available.

Specimen	No. of squares counted	No. of cells counted (λ)	$\frac{\sigma}{\sqrt{\lambda}}$	Platelet count (x 10 <sup>9</sup> /l)	CV (%)
A					
B					

Platelet count (x 10 <sup>9</sup> /l)	A	B
	λ	
By class count	σ	
	CV	
	$\bar{x}$	
By participant counts	SD	
	CV	
By electronic counter		
Method:		

**Exercise 19: Reticulocyte count**

Make reticulocyte preparations from the blood provided and carry out a reticulocyte count. The total RBC will be given. Report your results as a percentage and as an absolute count.

Number of fields examined		
Average number of red cells per field (Determined from 10-15 fields)		
Approximate total number of red cells surveyed		
Total number of reticulocytes counted		
Reticulocyte percentage		
RBC ( $\times 10^{12}/l$ )		
Absolute reticulocyte count		

Calculate the following:

Individual participant		
Retic %		
Range of $2\sigma$		
Class count		
Retic %		
Range of $2\sigma$		
Participant counts		
Retic %		
Range of 2 SD		
CV of test		

**Exercise 20: Interlaboratory quality control (external quality assessment)**

For this exercise, each student will be regarded as an independent laboratory. Use the data from Exercises 15, 16 and 18: calculate  $\bar{x}$  and SD; then calculate the deviation index (DI) as

$\frac{x - \bar{x}'}{SD'}$  where  $\bar{x}'$  and  $SD'$  are the mean and SD, respectively, after adjusting the original  $\bar{x}$  and SD by eliminating outliers.

By this procedure results from each individual can be compared with the consensus results from all participants. Performance is assessed as follows:

DI	<0.5	Excellent
	0.5-1.0	Satisfactory
	1.0-2.0	Acceptable but borderline
	>2.0	Unsatisfactory

Repeat the calculation of DI using median ( $m$ ) instead of mean ( $\bar{x}$ ).

SUPPLEMENTARY EXERCISES

The exercises which are described below are intended to illustrate the principles of standardization, when the calibration graph is non-linear (see Exercise 10). This is demonstrated by prothrombin time in which a local thromboplastin can be related to the WHO international standard by an appropriate procedure.

Exercise 21: International sensitivity index (ISI)

WHO has established this index in order to be able to relate measurements of prothrombin-time with any batch of thromboplastin to the primary standard. The ISI of a working preparation depends on the slope of the line of the calibration graph. This slope can be calculated accurately from the formula:

$$\text{Slope} = m + \sqrt{m^2 + 1}, \text{ where}$$

$$m = \frac{\sum(Ly - \bar{Ly})^2 - \sum(Lx - \bar{Lx})^2}{2\sum(Ly - \bar{Ly})(Lx - \bar{Lx})}$$

where  $Ly$  = log PT for each plasma with the RP  
 $Lx$  = log PT for each plasma with the WP  
 $\bar{Ly}$  = mean of log measurements of y  
 $\bar{Lx}$  = mean of log measurements of x

When the slope has been determined, the ISI of the working preparation is then calculated as follows:

$$\text{ISI of RP (as provided)} = \text{ISI (RP)}$$

$$\text{Slope} = b$$

$$\text{ISI of WP} = \text{ISI (RP)} \times b$$

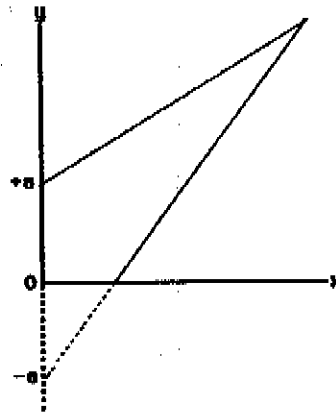
This is a complex formula. A simpler method is described below which is adequate for the laboratory wishing to calibrate a "home-made" thromboplastin for local use BUT NOT FOR THE MANUFACTURER OF A REFERENCE PREPARATION.

Exercise 22: Thromboplastin calibration graph

Results of prothrombin tests (secs):

Test	Reference preparation (y)	Working preparation (x)
1	13	13
2	13	16
3	15	17
4	17	19
5	17	21
6	13	15
7	28	35
8	30	40
9	30	43
10	35	46
11	36	50
12	48	70
13	50	90
14	40	50

- (1) Plot xy data on double-log graph paper, with reference preparation (y) on vertical axis and working preparation (x) on horizontal axis. Identify intercept of x on the y axis (= a). This may be positive or negative.



- (2) Calculation of slope (b) from formula  $y = a + bx$ :  $b = \frac{\bar{L}_y \times L_a}{\bar{L}_x}$

where  $\bar{L}_y$  = mean of logs of RP

$\bar{L}_x$  = mean of logs of WP

$L_a$  = log intercept of x on y (by calculation or from graph)

- (3) Calculation of slope from graph

Read off paired xy plots and intercept a on the graph.  
Convert to logs.

Calculate the slope =  $\frac{\text{Log } y_2 - \text{Log } y_1}{\text{Log } x_2 - \text{Log } x_1}$

Try this calculation at 4 different intervals to check reproducibility.

	1	2	3	4
: .....	:	:	:	:
: Log y	:	:	:	:
: .....	:	:	:	:
: Log x	:	:	:	:
: .....	:	:	:	:
: Slope	:	:	:	:
: .....	:	:	:	:

Compare results with the slope obtained with the formula given in the previous exercise.

Exercise 23: Standardization of prothrombin time test

You are provided with a working preparation (WP) of thromboplastin and a sample of thromboplastin standard or reference preparation (RP) with a stated International Sensitivity Index (ISI). You also have plasma samples from two normal subjects (N) and six patients stabilized on oral anticoagulant treatment (OAC).

Carry out prothrombin time tests (PT) on all the plasmas in duplicate with both the thromboplastins, in the following order:

<u>Test</u>	<u>Plasma</u>	<u>Thromboplastin</u>	<u>Test</u>	<u>Plasma</u>	<u>Thromboplastin</u>
1	N1	RP	9	OAC4	RP
2	N1	WP	10	OAC4	WP
3	OAC1	WP	11	OAC5	WP
4	OAC1	RP	12	OAC5	RP
5	OAC2	RP	13	OAC6	RP
6	OAC2	WP	14	OAC6	WP
7	OAC3	WP	15	N2	WP
8	OAC3	RP	16	N2	RP

Prepare a calibration graph as in Exercise 22

Record the mean times of each pair of tests. Plot these on double-log graph paper with the PT for RP on the vertical axis (y) and the PT for WP on the horizontal axis (x). Draw the best fit of a straight line to obtain a graph by means of which a PT obtained with the WP can be converted to the equivalent time for the RP. Calculate the slope and the ISI of the working preparation (WP).

Exercise 24: Measurement of prothrombin time

Carry out prothrombin time tests (PT) on the two plasmas A and B and on the pooled normal plasma provided, using the working preparation of thromboplastin which was standardized in Exercise 23. Record the means of duplicate tests. Convert the PT measurements to standardized PT (i.e. on the international scale) by means of the graph from Exercise 23.

Compare the results with those obtained by calculation by the formula  $x = by$ , where:

$x$  = log of standardized PT

$y$  = log of PT as measured

$b$  = ISI of working preparation (see Exercise 21)

Thus,

Standardized PT = Antilog of [log of PT (measured) + log of ISI].

	Plasma A	Plasma B	Normal plasma
PT - first measurement (seconds)			
PT - second measurement (seconds)			
Mean PT (seconds)			
y (= log PT)			
b (= log ISI)			
x = b + y			
Antilog x = standardized PT			
Standardized PT from graph			

**Exercise 25: Measurement of prothrombin ratio (PR) and international normalized ratio (INR)**

From the measurements obtained in Exercise 24 calculate the prothrombin ratio on the two plasmas as follows:

	Plasma A	Plasma B
Mean PT of test sample		
Mean PT of normal plasma pool		
Prothrombin ratio (PR) =		
$\frac{\text{PT of test plasma}}{\text{PT of normal plasma}}$		
INR* = PR <sup>ISI</sup>		

\* Obtain with calculator which has an  $x^y$  function or use the formula INR = Antilog of (log PR x ISI).

PART 4: PREPARATION OF CALIBRATION AND CONTROL MATERIALS

1. INTRODUCTION

Detailed instructions are given below for the preparation of calibration and control materials. The staff of a central laboratory must be able to prepare these for use in a national scheme. At least some of the general participants will also be expected to prepare a lysate, preserved blood specimens and preparations of fixed red cells and white cells, as these materials are required in practice in all laboratories for internal quality control of blood counts. In the organization of a national or area scheme, it may be practical for larger district laboratories to provide quality control material for smaller units in their districts. Accordingly, at least some of the course participants will be expected to accept this responsibility and must thus be familiar with the preparation of the quality control materials.

Some of these preparations are required for the exercises described in Part 3; accordingly organizers of training courses must be able to prepare small batches of the various materials for use by the course participants.

2. GENERAL NOTES

1. Human blood for use as calibration and control material for blood counts should be HBsAg and HIV antibody negative. Anticoagulated blood is usually available from Blood Transfusion Services; the anticoagulant is either citrate-phosphate-dextrose (CPD) or acid citrate dextrose (ACD-NIH A)<sup>1</sup>. For lysates and fixed RBC preparations blood in other anticoagulants (e.g. EDTA or heparin) can also be used.
2. In the following procedures care should be taken at all stages to avoid contamination. Where possible sterile glassware and reagents should be used and aseptic handling procedures observed.
3. Penicillin and streptomycin are added to aid sterility, e.g. 10<sup>6</sup> units benzyl penicillin and 1 g streptomycin sulphate per 500 ml material has been found to be satisfactory. As these compounds may cause allergic reactions they must be handled with care, avoiding inhalation or skin contact.

3. PREPARATION OF HAEMOLYSATE

1. Centrifuge anticoagulated blood in bottles of appropriate size (e.g. 30 ml screw-cap glass containers). Remove the plasma and buffer coat aseptically.
2. Add to each red cell deposit an excess of physiological saline (9 g/l NaCl), mix well, and re-centrifuge. Discard the supernatant and any remaining "buffy coat".
3. Repeat saline wash two times to ensure complete removal of plasma, white cells and platelets, each time removing the top layer of packed red cells.
4. Add to the washed cells half their volume of carbon tetrachloride, cap the containers and then shake vigorously on a mechanical shaker or vibrator for one hour. Refrigerate overnight to allow the lipid/cell debris to form a semi-solid interface between carbon tetrachloride and lysate.
5. On the following day centrifuge at about 2500 g for 20 minutes. Remove the upper lysate layers and pool them in a clean bottle.

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<sup>1</sup> CPD: Trisodium citrate, dihydrate 26.3 g; citric acid, monohydrate 3.27 g; sodium dihydrogen phosphate, monohydrate 2.22 g; dextrose 25.5 g; water to 1 l. The solution is sterilised by autoclaving at 126 °C for 30 minutes. Its pH is 5.6-5.8. For use, 7 volumes of blood are added to 1 volume of solution.

ACD (NIH-A): The following formula was recommended by the United States National Institute of Health; hence referred to as "NIH-A" solution. Trisodium citrate, dihydrate 22 g; citric acid, monohydrate 8 g; dextrose 25 g; water to 1 l. The solution is sterilised by autoclaving at 126 °C for 30 minutes. Its pH is 5.4. For use, 10 volumes of blood are added to 1.5 volumes of solution.

6. Using Whatman No. 1 filter paper in a Buchner funnel, filter the pooled lysate into a side arm flask connected to gentle water pump suction.
  7. Repeat filtration using Whatman No. 42 filter paper, changing the paper if filtration slows down. It is important not to overload the funnel with lysate.
  8. To each 70 ml of lysate add 30 ml of glycerol. After the addition of antibiotics (see item 2, point 3, page 48), this stock material may be stored at 4 °C until required for dispensing.
  9. If a lower concentration is required add an appropriate volume of 30% (v/v) glycerol in 9 g/l NaCl to the concentrated lysate. Mix well.
  10. With continuous mixing, dispense aseptically into sterile containers. Cap and seal.
  11. Method for assigning value of Hb concentration: The ICSH reference method described in the WHO document, "Recommended method for the determination of the haemoglobin content of blood" (LAB/84.10) is available on request. The CV should be less than 2%. Stored at 4 °C the product should maintain its assigned value for several months.
4. PREPARATION OF PRESERVED BLOOD
- Human blood is collected in blood collection bags containing CPD or ACD from donors of the same ABO blood group. Equine blood may be collected in bags of up to 2 l capacity.
1. Run the blood through blood administration sets directly into a round-bottom mixing flask and continue mixing for at least 20 minutes after the addition of the last unit of blood or other material.
  2. Cell levels may be adjusted, as follows:
    - a) To increase red cell count - sediment cells over exit vents of bag and run into the flask with minimum of plasma.
    - b) To lower red cell count - add solution of anticoagulant in 9 g/l NaCl; the anticoagulant:saline ratio must be the same as the usual anticoagulant: blood ratio (see item 2, point 1, page 48).
    - c) To lower white cell count - pass blood through a leucocyte filter (e.g. Sepacell R-500, Ashahi Medical Co. Ltd., Tokyo, Japan).
    - d) To increase white cell count - add fixed avian cells (see item 6, page 47: Preparation of pseudo white cells).
  3. Add antibiotics (see item 2, point 3, page 48).
  4. With continuous mixing dispense in sterile containers; cap and seal. Refrigerate at 4 °C until needed.
  5. For analysis, the sample should be gently mixed on a roller mixer or by hand before opening. Unopened vials of human blood keep in good condition for about three weeks at 4 °C, and those of equine blood for up to three months.
  6. Methods for assigning values
    - a) RBC: Electronic counting - at least 10 replicate counts on each of two vials from the batch by calibrated electronic counter, or by careful haemocytometry; dilution by precalibrated pipettes. The CV should be less than 2% when the count is done by electronic counter.
    - b) PCV: ICSH method (Journal of Clinical Pathology, 1980, Vol. 33, pp. 1-2). The CV should be less than 2%.

## 5. PREPARATION OF FIXED RED CELLS

### Reagents

#### 0.15M iso-osmotic phosphate buffer (pH 7.4)

- (A) - 23.4 g/l sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ )
- (B) - 21.3 g/l anhydrous disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) or 53.7 g/l  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ .

Both stock solutions keep well when refrigerated.

For use, mix in proportion 18 ml A + 82 ml B. Check that the pH is 7.4.

#### 0.25% glutaraldehyde fixative

To 1 litre of phosphate buffer, add 5 ml 50% glutaraldehyde solution (commercially available), mix and use at once.

#### Glycerol-saline

Add 60 volumes of glycerol to 40 volumes of 9 g/l NaCl. Store in a refrigerator.

#### Antibiotic

Penicillin-streptomycin (see item 2, point 3, page 48).

### Method

1. Centrifuge blood. Remove the plasma.
2. Add an excess of phosphate buffer to the red cells, mix and transfer to centrifuge bottle; re-centrifuge and discard supernatant and buffy coat.
3. Repeat wash and centrifugation twice.
4. Add to the washed cells 10 times their volume of glutaraldehyde fixative, mix by vigorous shaking to ensure complete resuspension and rotate slowly on a mechanical mixer for one hour. To test for complete fixation, centrifuge 2-3 ml of the suspension, discard supernatant, add 2-3 ml water to the deposit, mix and centrifuge; if haemolysis occurs, fixation is incomplete. Either more time is needed or the stock glutaraldehyde requires replacement.
5. When fixation is complete, centrifuge the suspension and discard supernatant.
6. Add an excess of distilled water to the fixed cell deposit, resuspend and mix by stirring and shaking: again centrifuge and discard supernatant. Repeat twice.
7. Resuspend the washed fixed cells to approximately 30% concentration in 0.1% sodium azide in 9 g/l NaCl. Mix well by vigorous shaking.
8. If the material has been stored, re-suspend by vigorous hand shaking (or by a vortex mixer) until no clumps remain at the base of the container, then roller mix for at least 20 minutes. If available, sonication for 1-2 minutes should also be used.
9. Carry out a rough count by routine method to determine the approximate concentration.
10. Autoclave at 121 °C for 15 minutes.
11. For use, mix by vigorous hand shaking or by vortex mixer followed by roller mixing for at least 20 minutes.

12. Dilute in glycerol-saline to the required concentration.
13. If the material has been stored, resuspend by vigorous hand shaking and/or by vortex mixing or sonication followed by mechanical mixing for at least 15 minutes. Transfer to mixing unit and mix for at least 20 minutes before dispensing with continuous mixing into sterile containers, each of which contains two or three 3 mm glass beads to assist sample resuspension. Cap and seal.
14. For analysis, resuspend by vigorous hand shaking or vortex mixing and place on a mechanical mixer for at least 15 minutes before opening the tube.  
  
Undiluted fixed cell preparations are not suitable for use in fully automated counting systems.
15. Method for assigning values to material (particles concentration): see item 4, point 6, page 49. At 4 °C the material should be stable for at least one year.

#### 6. PREPARATION OF "PSEUDO-WHITE" CELLS

Chicken and turkey red blood cells are nucleated and, when fixed their size, as recognized by electronic cell counters, is within the leucocyte size range on these counters. Thus, they are suitable to act as "pseudo-white" cells in preserved whole bloods. For this purpose it is sufficient to collect 25 ml of blood into ACD (NIH-A) and process as for fixed red cells (section 5, page 50). This quantity of blood is sufficient for many preparations. An appropriate amount is added to a bulk of preserved blood.

1. If the material has been stored, resuspend by vigorous hand shaking (or by a vortex mixer) until no clumps remain at the base of the container, and follow by mechanical mixing for at least 20 minutes.
2. Carry out a rough count by routine method to determine the approximate concentration.
3. Transfer an appropriate amount to a volume of preserved blood (see section 4, page 49) from which the leucocytes have been filtered.
4. Mix well for 20 minutes and with continuous mixing dispense into sterile containers. Cap and seal.
5. For analysis resuspend by vigorous hand shaking followed by leaving on a mechanical mixer for at least 15 minutes before opening the tube.
6. Method of assigning WBC value: Haemocytometry on at least 10 replicate counts from each of two vials from the batch; dilution by precalibrated pipettes.

The CV should be less than 5%. At 4 °C the shelf life will be several months, unless the medium becomes infected.

#### 7. PREPARATION OF FIXED PLATELETS

##### Reagents

EDTA solution. EDTA (100 g/l) in modified Alsever's buffer. This will keep at 4 °C for up to 6 months.

Modified Alsever's buffer. (A) Trisodium citrate 16 g; NaCl 8.2 g. Make up to 1 litre with distilled H<sub>2</sub>O. Keep at 4 °C. (B) Dextrose 41 g. Make up to 1 litre with distilled H<sub>2</sub>O. Keep at 4 °C. Mix A + B immediately before used. Discard any remaining solution after use.

Fixative. Formaldehyde (40% v/v) 2 ml per 100 ml of Alsever's buffer.

Platelet-rich plasma. Collect one or more units of blood into plastic bags with ACD or CPD anticoagulant (see page 48). Centrifuge the bags at 200 g for 10 minutes; collect the platelet concentrated from each into a transfer pack or another plastic bag. When reconstituted (see below) one unit of normal blood should provide 500 ml with a platelet count of about  $70-80 \times 10^9$  per litre or 250 ml with a platelet count of  $150 \times 10^9$  per litre.

#### Method

- (1) Add one unit of platelet-rich plasma into each of a series of 150 ml glass bottles containing 1 ml of EDTA solution. Leave at 37 °C in a water-bath for 1 h to allow the platelets to disaggregate.
- (2) If possible the disaggregation should be checked by passing a small sample from each bottle through a blood counting system set for platelet counts and with a facility for analysing size-distribution curves, or by visual inspection of a diluted sample in a haemocytometer chamber. If aggregation is still present leave in the water-bath for another hour.
- (3) Dispense 200 ml of fixative into each of a series of plastic bottles, and into each add the solution from one of the glass bottles. Leave at room temperature (ca 20 °C) for about 48 hours.
- (4) Centrifuge the bottles at ca 50 g for 10 minutes at room temperature.
- (5) Distribute the platelet-rich supernatants from the plastic bottles equally into two sterile 500 ml glass bottles. Fill the bottles with Alsever's buffer.
- (6) Wash three times in Alsever's buffer, centrifuging between washes at ca 750 g for 30 minutes at room temperature. After the third wash remove the buffer and resuspend the platelets in 10 ml of fresh buffer. Mix well.
- (7) Pool the contents of the two bottles into a glass container or mixing flask.
- (8) Add antibiotic (see item 2, point 3, page 48) and with continuous mixing dispense 5 ml volumes into sterile plastic containers. Cap and seal.
- (6) Method for assigning values for platelet count: Haemocytometry on at least 5 replicate counts on each of two vials from the batch.

The CV should be less than 5%. The material will be satisfactory for at least one year if stored at 4 °C.

APPENDIX I

DEFINITIONS USED BY THE INTERNATIONAL COMMITTEE FOR STANDARDIZATION IN  
HAEMATOLOGY

1. STANDARDS

1.1 ICSH standards: These include materials and physical devices, methods and procedures, calibration and controls for equipment and for test procedures, systems of nomenclature and classification and modes of practice which have been established as standards by ICSH by consensus in accordance with defined procedure.

1.2 Reference material: A material or substance, one or more properties of which are sufficiently well established to be used for the calibration of an apparatus, the assessment of a measurement method or for assigning values to other materials. Where possible, it must be based on or traceable to a metrological standard.

1.3 International biological standards are reference standards which cannot be determined by exactly defined physical or chemical methods, but to which have been assigned international units of activity as defined by the World Health Organization. These materials are not intended to be used in the laboratory working procedures but serve as the means by which national and commercial reference materials and calibrators can be controlled.

1.4 Certified reference material (CRM): A reference material with one or more values assigned by means of a collaborative study in several designated centres in accordance with a specified protocol, controlled by a certifying body. In the case of an ICSH reference material, the certifying process is directed to the secretariat on behalf of the ICSH Board, advised by an appropriate Expert Panel.

2. MATERIALS AND METHODS

2.1 Definitive method: A method which after exhaustive investigation is found to have no known source of inaccuracy (see 2.8) or ambiguity. It will, however, have a (known) degree of imprecision (see 2.11) which should be stated.

2.2 Reference method: A clearly and exactly described technique for a particular determination which, in the opinion of a defined authority, provides sufficiently accurate and precise laboratory data for it to be used to assess the validity of other laboratory methods for this determination. The accuracy of the reference method must be established by comparison with a definitive method where one exists.

An international reference method is one that has been established by a defined international authority.

A reference reagent is one which has defined and clearly described properties; it is used with a reference method or procedure, or, when appropriate, in conjunction with an international reference standard.

2.3 Selected method: A procedure the reliability of which has been validated by a collaborative study and which is recommended by a defined authority for routine use in laboratory analysis, having been selected on the grounds of its accuracy and precision, the intended scope of the test, economy of labour and materials, and ease of operation.

2.4 Diagnostic kit: A package containing reagents, working standards and/or other material and a method protocol designed for reliable performance of a specified analysis or analyses.

2.5 Calibrator: A substance or device used to calibrate, graduate or adjust a measurement. It must be traceable to a reference standard.

2.6 Calibration: The determination of a bias conversion factor of an analytical process under specified conditions, in order to obtain accurate measurement results. The accuracy over the operating range must be established by appropriate use of reference methods, reference materials and/or calibrators.

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2.7 Accuracy: A measure of agreement between the estimate of a value and the true value. Accuracy has no numerical value; it is measured as the amount of (degree of) inaccuracy.

2.8 Inaccuracy: Numerical difference between the mean of a set of replicate measurements and the true value. This difference (positive or negative) may be expressed in the units in which the quantity is measured, or as a percentage of the true value.

2.9 Bias: Systematic factor resulting in inaccuracy.

2.10 Precision: Agreement between replicate measurements. It has no numerical value but is recognized in terms of imprecision.

2.11 Imprecision: Standard deviation or coefficient of variation of the results in a set of replicate measurements.

2.12 Value: Measurable quantity of an analyte; it is used in a compound term.

True value is obtained by a definitive method.

Reference value is the best available estimate of true value, as obtained by a reference method.

Assigned value is the value assigned either arbitrarily (e.g. by convention) from results obtained using a selected method or from results using methods with unknown bias.

2.13 Comparability: Correlation of results of a measurement by an analytic procedure with results selected as reference values.

2.14 Control: A substance, device or procedure used for checking that the performance of an analytic instrument as process is constant. When a substance is used in this context in routine practice for checking the concurrent performance of an analytic process or instrument, it must be similar in properties to, and be analysed along with, patient specimens.

2.15 Specimen: Material available for analysis.

2.16 Sample: A representative part of a specimen which is used in the analysis.

2.17 Specificity: The ability of an analytical method to determine solely the component(s) it purports to measure.

2.18 Sensitivity: the ability of an analytical method to detect small quantities of the measured compound.

## 3. QUALITY ASSURANCE

3.1 Quality assurance programmes: All steps to be taken by the director of a laboratory to ensure reliability of laboratory results and to increase accuracy, reproducibility and between-laboratory comparability. This includes proficiency surveillance (see below), the constant use of internal quality control and participation in an external quality assessment scheme. It also includes participation in training courses, conferences, collaborative studies of instruments and laboratory methods and other cooperative activities intended for the improvement of laboratory performance. A quality assurance programme in haematology must also be concerned with clinical aspects of haematology.

3.2 Internal quality control: Internal quality control is the set of procedures undertaken in a laboratory for the continual assessment of work carried out within the laboratory and evaluation of the results of tests to decide whether the latter are reliable enough to be released to the requesting clinician. The procedures should include tests on control material and statistical analysis of patients' data. The main object is to ensure day-to-day consistency of measurement or observation, if possible in agreement with an agreed indicator of truth such as control material with assigned values.

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3.3 External quality assessment: External quality assessment refers to a system of retrospectively and objectively comparing results from different laboratories by means of surveys organised by an external agency. The main object is to establish between-laboratory and between-instrument comparability, if possible in agreement with a reference standard where one exists. External quality assessment schemes may be regional, national or international. They may also be limited to the users of a particular instrument.

3.4 Interlaboratory trials: Collaborative, comparative performance of tests by a group of laboratories to assess aspects of tests or materials including candidate reference materials. The data obtained by an external quality assessment scheme can be used as a starting point if they are analysed in terms of the test procedure, instrumentation or materials, rather than from the viewpoint of individual laboratory performance. This activity can provide guidance for a quality assurance programme.

3.5 Quality surveillance: Examination of laboratory practice by an independent body including (as appropriate) participation by government and professional associations with authority for corrective measures. Data obtained from an approved external quality assessment scheme should be used as the basis for identifying unsatisfactory performance.

3.6 Proficiency surveillance: Supervision and action to ensure good laboratory practice. An important aspect is internal quality control and participation in an external quality assessment scheme, but it also includes attention to proficiency in specimen collection and labelling, delivery of specimens to the laboratory, record keeping and reporting, environmental and storage effects on specimens, interpretation of test results and relevance of various tests for the clinical information required. It also includes maintenance and control of equipment and apparatus, staff training, and protection of staff health and safety.

APPENDIX 2

SYMBOLS AND ABBREVIATIONS USED IN THIS MANUAL

IQC	Internal quality control
EQA	External quality assessment
NEQAS	National EQA scheme
$\sqrt{\quad}$	Square root
$\Sigma$	Sum
SD	Standard deviation
$\sigma$	Count variance
$\lambda$	number of cells counted
$\bar{x}$	Mean (of set of results $x_1, x_2, x_3 \dots x_n$ )
m	Median
CV	Coefficient of variation
d	)
(x - $\bar{x}$ )	) Difference of measurement from mean

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