



WORLD HEALTH ORGANIZATION  
ORGANISATION MONDIALE DE LA SANTE

46060  
DISTR. : LIMITED  
DISTR. : LIMITEE

WHO/LBS/92.8

Copyright: World Health Organization. Reproduced with permission.

ENGLISH ONLY

## CALIBRATION AND MAINTENANCE OF SEMI-AUTOMATED HAEMATOLOGY EQUIPMENT

Prepared on behalf of the Unit of  
Health Laboratory Technology and Blood Safety  
World Health Organization  
Geneva, Switzerland

by

J.M. England  
Consultant Haematologist - Watford General Hospital  
Watford WD1 8HB - England

S.M. Lewis  
Emeritus Reader in Haematology and Senior Research Fellow  
Royal Postgraduate Medical School  
London W12 ONN - England

E. Lloyd  
Principal Scientist, Haematology Department - Royal Postgraduate Medical School  
London W12 ONN - England

R.M. Rowan  
Senior Lecturer in Haematology - Western Infirmary  
Glasgow G11 6NT - Scotland

The majority of central and regional haematology laboratories are likely, nowadays, to have relatively complex instruments. These include microscopes, photometers and spectrophotometers, micro-haematocrit centrifuges, autodiluters, blood cell counters, automated slide stainers, electrophoresis systems, coagulometers and basic equipment for hospital blood transfusion serology.

Skill and knowledge are required to use these various instruments correctly, to calibrate them and keep them in good working order. This document describes how they work and provides advice for their installation and calibration, routine daily care (especially in the unfavourable climatic environment which is present in many developing countries) and periodic service maintenance. A section on trouble-shooting provides sufficient detail to enable laboratory staff to identify common causes of malfunction and to carry out simple repairs. There is also a section on laboratory safety with rules for preventing and if necessary dealing with accidents caused by fire, electric shock, chemical contamination, mechanical injury, microbiological infections.

Although the document is intended primarily for central and regional laboratories, many district hospitals will have some of the apparatus which is described, while knowledge of the correct use and care of microscopes is also essential for health clinics where microscope work is undertaken.

This document is not issued to the general public, and all rights are reserved by the World Health Organization (WHO). The document may not be reviewed, abstracted, quoted, reproduced or translated, in part or in whole, without the prior written permission of WHO. No part of this document may be stored in a retrieval system or transmitted in any form or by any means - electronic, mechanical or other - without the prior written permission of WHO.

The views expressed in documents by named authors are solely the responsibility of those authors.

Ce document n'est pas destiné à être distribué au grand public et tous les droits y afférents sont réservés par l'Organisation mondiale de la Santé (OMS). Il ne peut être commenté, résumé, cité, reproduit ou traduit, partiellement ou en totalité, sans une autorisation préalable écrite de l'OMS. Aucune partie ne doit être chargée dans un système de recherche documentaire ou diffusée sous quelque forme ou par quelque moyen que ce soit - électronique, mécanique, ou autre - sans une autorisation préalable écrite de l'OMS.

Les opinions exprimées dans les documents par des auteurs cités nommément n'engagent que lesdits auteurs.

## CONTENTS

	<b>Page</b>
<b>INTRODUCTION</b> .....	3
<b>PART I: OPERATING PRINCIPLES, SELECTION AND CALIBRATION</b> .....	4
Chapter 1. Microscopy .....	4
Chapter 2. Photometers and spectrophotometers .....	13
Chapter 3. Microhaematocrit centrifuge .....	22
Chapter 4. Autodiluters .....	24
Chapter 5. Semi-automated blood cell counters .....	26
Chapter 6. Slide staining instruments .....	29
Chapter 7. Electrophoresis systems .....	32
Chapter 8. Coagulometers .....	36
Chapter 9. Hospital blood transfusion laboratory equipment .....	39
<b>PART II. CARE OF EQUIPMENT</b> .....	47
Chapter 10. Preventive maintenance and simple trouble shooting .....	47
Chapter 11. Safety in the haematology laboratory .....	64
References .....	76

## INTRODUCTION

Much of the equipment used in clinical laboratories is complex. It requires technical skill for correct use, and for maintenance. Failure to ensure regular or preventive maintenance leads to break-down and if an instrument does break down it may be difficult to get it repaired. Inadequate service by manufacturer and lack of ability by laboratory staff or hospital workshop to identify and repair minor faults frequently results in equipment lying idle for long periods and being discarded, often for want of relatively simple repair or replacement of a non-functioning component. Many laboratories do not have a stock of consumable items such as electric bulbs for microscopes or photometers.

In developing countries laboratories face further problems as a result of adverse climatic conditions, poor electricity supplies, lack of recommended reagents and calibration materials, instruction manuals which are not translated into the language of the country, are difficult to follow, and do not provide adequate guidance or "trouble-shooting".

In order to overcome these problems a system of maintenance is necessary. Day to day care of the instruments should be complemented with a regular programme of preventive maintenance within the laboratory. Staff should be trained to undertake simple repairs and replacement of defective components, and procedures should be adopted for calibration of the instrument which can be undertaken without need for commercial materials.

The aim of this document is to give guidance on these aspects in relation to equipment commonly used in haematology and blood transfusion. It is not intended to provide instruction on specific diagnostic tests, but test procedures are described where they are relevant to checking the function of an instrument and its calibration. This document should thus be used in conjunction with appropriate laboratory manuals. It is directed essentially to workers in central and regional laboratories, but undoubtedly many district hospitals, too, will have at least some of the equipment described. Furthermore, maintenance of microscopes in a good condition is essential for reliable morphology; so that this section is of importance to all persons who undertake microscopic work, including laboratory technicians at health centres.

There is an increasing awareness of the need for laboratory safety. A chapter is devoted to this important subject, with special reference to the risk of infection by biohazardous materials. This includes the need to ensure that instruments are safe to use and, when decontamination is required, that this will not damage any of their components nor affect their performance.

It was the intention that the technical details provided should be sufficient to enable the reader to undertake the work entailed without need to refer to other publications. There are, however, a number of other WHO documents which provide useful supplementary and complementary information. We have also drawn intensively on the recommendations of the International Council for Standardization in Haematology. A list of these documents and publications is given at the end of the document.

## PART I: OPERATING PRINCIPLES, SELECTION AND CALIBRATION

### CHAPTER 1. MICROSCOPY

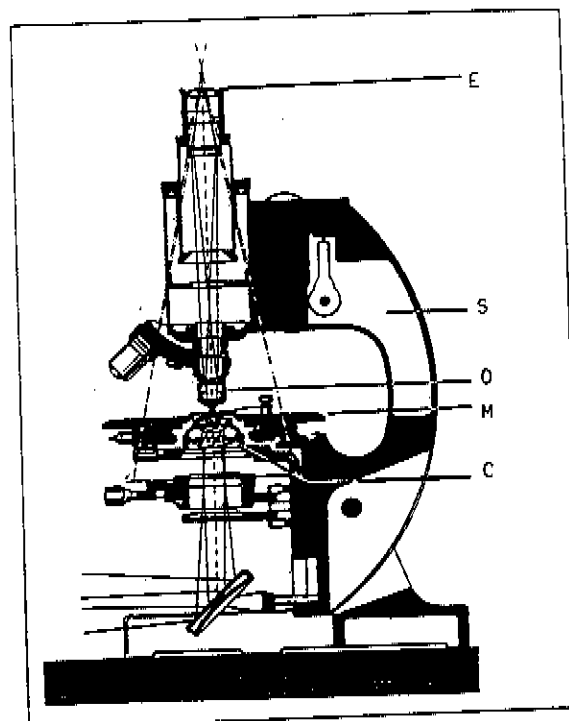
The microscope is one of the most important instruments in the hospital laboratory. It is used in haematology for examination of blood (and bone marrow) morphology; for counting chamber haemocytometry for WBC and platelet counts; for observing red cell agglutination in blood transfusion practice.

#### PRINCIPLE

The main features are illustrated in Fig. 1.1

There are three components:

- (1) stand
- (2) optical system
- (3) illumination system



**Fig. 1.1 - Microscope**

Cross-section of microscope showing its component parts and the light path.

E = Eyepiece; S = stand; O = Objective;

M = Stage; C = Condenser

- (1) The **stand** consists of (a) a base, (b) a tube which holds the lenses with a revolving nosepiece for changing the objectives (see below), (c) a support to hold the tube and containing adjustment screws to raise and lower the objectives for focussing onto the object and (d) a platform together with a mechanical stage to move the object slide.
- (2) The **optical system** consists of objectives and eyepieces.

### Objectives

There are usually three objectives which screw into the nosepiece. The common objectives are x10, x40 and x100 (previously known as 16 mm, 4 mm and 2 mm respectively). These figures are engraved on the sleeve of the lens, together with the numerical aperture (NA). As a rule the NA of the x10 objective is 0.25; of the x40 objective it is 0.65; and of the x100 objective it is 1.30. The higher the NA of the lens, the greater is its resolving power. Usually the x100 objective requires oil immersion.

### Eyepiece(s)

There may be one eyepiece (monocular microscope) or two (binocular microscope). They are inserted in the end of the tube distant from the objectives. Their magnifying power is marked on them; as a rule this is x6 or x10.

Magnification of the object is obtained by multiplying the magnifying power of the objective and the eyepiece. Thus:

Eyepiece	x10	x40	x100	
x6	60	240	600	} Final magnification } of object
x10	100	400	1000	

### (3) Illumination system

#### (a) Light source

Preferably the source of light should be a tungsten filament lamp. This may be reflected into the microscope by a mirror or provided directly by a low voltage (e.g., 6V) light bulb fitted into the base of the microscope. The latter requires a mains transformer or battery to provide the 6V power.

In the absence of an electricity source daylight can be used with a reflecting mirror, at least for lower magnifications; it is inadequate when using the x100 objective. Furthermore, the microscope must **not** be used in **direct** sunlight: if necessary a round flask of water placed in front of the

microscope or a grey-density or blue filter beneath the stage can be used to cut down the intensity of the light.

(b) Condenser

This is a large convex lens which converges the light on the object to be examined. It is situated between the light source and the stage, and can be raised for maximum illumination or lowered for minimum illumination.

(c) Iris diaphragm

The diaphragm, inside the condenser, is used to increase or reduce the amount of light that passes into the condenser.

### SELECTION OF INSTRUMENT

A binocular microscope has the advantage over a monocular that as the image can be seen with both eyes at once it is more restful for prolonged work. However, it requires brighter illumination. No more detail can be seen than with the monocular and it is significantly more expensive.

#### Objectives

x10 is useful in order to obtain an overall impression of cellularity in a bone marrow smear or in a section. It will also allow easy detection of filaria in a blood film. It is the correct magnification for examining wet preparations for red cell agglutination in blood transfusion work.

x40 is a general purpose objective for routine screening of films, differential leucocyte counts, recognition of morphologically abnormal cells and detection of malaria and other blood parasites.

x100 is necessary to see small inclusions in red cells (e.g., Howell Jolly bodies), to identify the type of malaria parasite, to determine morphological characteristics of leucocytes, etc. Using x100 is much slower than using x40 in routine practice; it requires oil-immersion<sup>1</sup> and a good light source.

---

<sup>1</sup> But when a dry (non-oil) objective is used, it is essential to cover the specimen with a coverglass in order to have a good resolution of details. For this one can use immersion oil as a temporary mount, or neutral mountant as a permanent mount.

## ADJUSTMENT (CALIBRATION)

### 1. Illumination

Place a slide (and cover-glass) on the stage, position the x10 objective, and carry out the following procedures:

#### (a) Centering light source

##### With mirror

Use the plane side of the mirror<sup>2</sup>. Open the iris diaphragm to the maximum. Raise the condenser. Place a piece of thin white paper over the lens at the top of the condenser. This piece of paper should show an image of the electric bulb, surrounded by a circle of light. Adjust the mirror so that the image of the bulb is in the exact centre of the circle of light. If daylight is being used adjust the mirror so that the brightest part of the light is in the centre.

##### With built-in light

Centre the light by means of adjusting screws to obtain the same result as described above.

#### (b) Centering condenser

Lower the condenser. Open the iris diaphragm. Examine the slide with the x10 objective. Bring the object into focus. Close the diaphragm: a blurred circle of light surrounded by a dark ring appears in the field. Raise the condenser slowly until the edges of the circle of light are in sharp focus. Adjust the position of the mirror (if necessary) so that the circle of light is in the exact centre of or superimposed upon the bright area surrounded by the dark zone. Using the centering screws of the condenser, adjust so that the circle of light is in the exact centre of the field.<sup>3</sup>

---

<sup>2</sup> When the condenser is used, which is generally all the time, only the flat mirror should be used. The curved mirror is itself a form of low power condenser, and it must never be used together with a condenser.

<sup>3</sup> In some microscopes it is not the objective holder but the stage that is moved up or down by the coarse and fine adjustment screws. In this case the screws must be turned in the opposite direction to bring the image into focus.

(c) Adjustment of diaphragm

Open the diaphragm completely. Remove the eyepiece and look down the tube: the upper lens of the objective will be seen filled with a circle of light. Close the diaphragm slowly until the circle of light takes up only 2/3 of the surface.

2. Focusing the object

(a) Using a low-power objective (x10)

Rack the condenser down to the bottom. Lower the objective until it is just above the slide preparation. Raise the objective, using the coarse adjustment screw, until a clear image is seen in the eyepiece.

Occasionally, although the objective has been lowered as far as possible, a clear image cannot be obtained: this is because the fine adjustment screw has been turned right to the end. Turn it back as far as it will go in the other direction and then focus by raising the objective.

If there is insufficient illumination rack up the condenser slightly.

(b) Using a high-power objective (x40)

Rack the condenser half way down. Lower the objective until it is just above the slide preparation. Using the coarse adjustment, raise the objective very slowly until a blurred image appears of the field. Bring into focus using the fine adjustment. Raise the condenser to obtain sufficient illumination.

(c) Using the oil-immersion objective (x100)

Perfectly dry, stained preparations must be used. Place a tiny drop of immersion oil on the part to be examined (use synthetic oils, which do not dry, in preference to cedar wood oil, which dries quickly). Rack up the condenser as far as it will go. Lower the x100 objective until it is in contact with the oil. Bring it as close as possible to the slide, but avoid pressing on the preparation. Look through the eyepiece and turn the fine adjustment very slowly until the image is in focus.



### 3. Slides and coverglasses

The working distance of the objective is the distance between the objective and the object to be visualized. The greater the magnifying power of the objective, the smaller the working distance. If the coverglass is too thick it will not be possible to focus at high magnification.

Objective	Working distance
x10	5-6 mm
x40	0.5-1.5 mm
x100	0.15-0.20 mm

Thus, the cover-glass should be no more than 0.15 mm thick for examination of covered preparations by the x100 oil-immersion lens. Obviously, preparations which are not covered can be examined under oil immersion without this problem.

If the glass slide is too thick, this may prevent correct focus of the light path through condenser to the object.

### 4. Oil-immersion

When a beam of light passes from air into glass, it is bent, and when it passes back from glass to air, it is bent back again to its original direction. This has little effect on low power objectives, but with high power lenses this bending limits not only the amount of light which can enter the lens but also its resolving power. This bending effect, and its limitations on the objective, can be avoided by replacing the air between the specimen and the lens by an oil which has the same optical properties as glass.

In this way, instead of the light bending as it passes from glass to air and then again as it passes back to glass, it passes in a straight line from glass through the oil and back to glass, as though it were passing through glass all the way.

### 5. Glare

The above sections describe the way in which the light must be adjusted and the microscope focussed. Failure at any stage in this procedure will result in glare which interferes with the production of a good image. The following causes of glare must be avoided:

- (a) Stray light entering the eye from outside the microscope (for example from windows or other sources of light in the room). This external glare can be reduced by using the microscope in a subdued light and preferably in a darkened part of the room. When this cannot be done, an eye shade helps to exclude such glare.

- (b) A larger source of illumination than is necessary (one that illuminates more of the object than is seen in the field of view). This illumination glare can be avoided by using a light source which illuminates no more than the field of view. This needs a different size of light source for each objective, large for the lowest power, and small for the highest.
- (c) A larger condenser aperture than is necessary (one which more than fills the objective with light). This condenser glare can be reduced by increasing contrast in the microscope, but this is at the expense of resolving power, and if the other sources of glare are reduced, the condenser aperture does not have to be reduced so much.
- (d) Reflection back and forth in the specimen between slide and cover glass or in air between specimen and the front of the objective may cause glare which can be reduced by selecting an appropriate mountant such as immersion oil, Canada Balsam or neutral mountant. If a cover glass can be omitted and replaced by the use of the oil immersion lens, some reflections can be avoided and glare is further reduced.
- (e) Stray light from the interior of the objective, microscope tube or eyepiece requires help from the manufacturer or a microscope expert.

## **ROUTINE MAINTENANCE**

The microscope is a delicate instrument which must be handled gently. It must be installed in a clean environment, away from chemicals, direct sunlight, heating source or moisture. If the stage is contaminated with saline (in blood transfusion work), it must be cleaned immediately to avoid corrosion. Humidity and high temperatures cause growth of fungus which can damage optical surfaces. As storage in a closed compartment encourages fungal growth, rather than keeping it stored in its wooden box it is generally better to keep it standing in place ready for use, but protected by a light plastic cover. In humid climates it may be necessary to use a drying agent, e.g., calcium chloride in a small container.

### **Cleaning of the microscope**

#### **Optics**

After use the immersion objective should be wiped with lens tissue, absorbent paper, soft cloth or medical cotton wool.

Other lenses (objectives and eyepieces) which are smeared with oil should be wiped with a very little xylol or toluene or the following cleaning solution:

petroleum ether	40%
ethanol	40%
ether	20%

The lenses must **not** be soaked in alcohol as this may dissolve the cement. Contamination of the non-optical parts can be removed with mild detergent; grease and oil are removed with petroleum ether followed by 45% ethanol in distilled water.

The eyepieces must be cleaned from dust with a blower or soft camel hair brush. If dust is inside the eyepiece unscrew the upper lens and clean the inside with the blower or the soft brush.

#### Condenser and iris

The condenser is cleaned in the same way as the lenses with a soft cloth or tissue moistened with xylol or toluene. The mirror is cleaned with a soft cloth moistened with 96% alcohol. The iris diaphragm is very delicate and if damaged or badly corroded it is usually beyond repair.

#### Mechanical parts

The microscope controls must never be forced. If any of the controls begin to run hard, a touch of machine oil may be required. This must be proper machine oil, as vegetable oils become dry and hard. This procedure applies to coarse adjustment, fine adjustment, condenser focusing and mechanical stage. It is recommended periodically to clean and give a touch of oil to all accessible movements. This lubrication not only keeps the parts running smoothly in use but reduces wear and protects the parts against corrosion. The surface of the fixed stage must be kept dry, for if a slide is wet underneath it will be difficult to move, and the increase of pressure put on it can damage the mechanical stage if this is forced.

#### Additional precautions in hot climates

##### (a) Hot humid climates

In hot humid climates, if no precautions are taken, fungus may develop on the microscope, particularly on the surface of the lenses, in the grooves of the screws and under the paint, and the instrument will soon be useless. This can be prevented as described below.

i. Laboratory with electricity

Every evening place the microscope in a warm cupboard. This is a cupboard with a tight-fitting door, heated by one or two 40-watt light bulbs (for a cupboard just big enough to take 1-4 microscopes one bulb is enough). The bulb is left on continuously, even when the microscope is not in the cupboard. Check that the temperature inside the cupboard is at least 5°C warmer than that of the laboratory.

ii. Laboratory without electricity

The microscopes should always be kept in the open air, in the shade, but near a sunny spot. Never put a microscope in its wooden box (even overnight) but always use a cover. The microscope must, however, be cleaned daily to get rid of dust.

(b) Hot dry climates

In hot dry climates the main problem is dust. Fine particles work their way into the threads of the screws and under the lenses. This can be avoided as follows:

- i. Always keep the microscope under an dustproof plastic cover when not in use.
- ii. At the end of the day's work, clean the microscope thoroughly by blowing air on it from a rubber bulb.
- iii. Finish cleaning the lenses with a lens brush or fine paintbrush. If dust particles remain on the surface of the objectives, remove with clean paper.
- iv. If there is a wet season lasting more than a month, take the precautions recommended above for hot humid climates.

**PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING (see Chapter 10)**

## CHAPTER 2. PHOTOMETERS AND SPECTROPHOTOMETERS

A photometer is an optical instrument which is used for measurement of light absorption. According to the Lambert-Beer's law the degree of light absorption is related to the concentration of the compound in solution. The photometer or spectrophotometer is an essential instrument in the laboratory; it is used for a number of analytic tests. In haematology it is most commonly used for measuring haemoglobin.

Instruments with a tungsten light source and filters for wavelength selection are usually referred to as *photometers* or in a simpler version as *colorimeters*, while *spectrophotometers* are more complex instruments which can be used to determine concentration, measure absorbance at specified wavelengths and record the absorption spectrum.

Spectrophotometers may be of the single or double beam type. In the single beam instrument light passes from the source through the wavelength selector and then via a sample cuvette through to the detector. In the double beam instrument light passes through a beam splitting device and then via separate sample and reference cuvettes to separate detectors. Performance of spectrophotometers varies according to the range of wavelengths and the degree of selection.

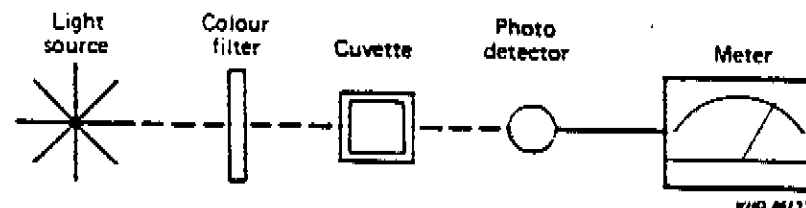
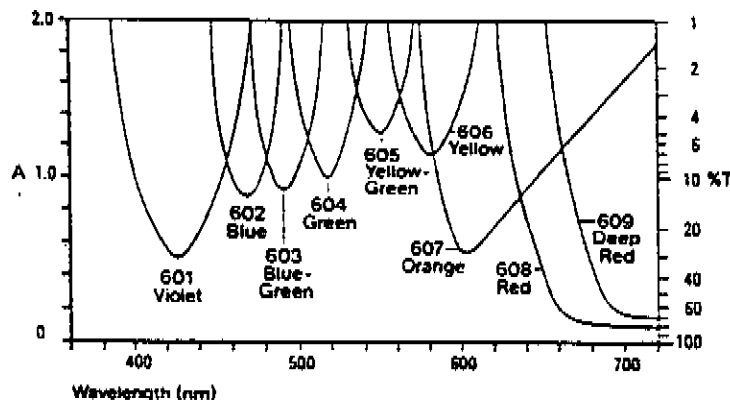


Fig. 2.1  
Schematic diagram of a photometer

### COMPONENTS OF A PHOTOMETER

The main features of a typical instrument are illustrated in Fig. 2.1. The light source is usually a tungsten filament lamp which, when provided with a constant voltage, emits white light of constant intensity. The colour filter is used to select light of a colour which is absorbed by the solution. This colour is expressed in terms of the wavelength (in nanometers, nm) of the light, and for the visible spectrum extends from about 400 nm (violet) to 700 nm (red). The filter or wavelength used must be carefully selected so that the colour of the light it transmits is maximally absorbed by the solution. Thus a blue filter (transmitting light at 400-450 nm) is best for a red coloured solution which absorbs blue light. The efficiency of a filter depends on the band width of the wavelengths it transmits. Commonly used glass-gelatin filters (Fig. 2.2) have a wider wave-band (up to 50 nm) than interference types (about 10 nm). Photometers or colorimeters used for a specific function (e.g., haemoglobinometers) have a built-in filter with a narrow band width.



**Fig. 2.2**  
**Transmission curves of filters**  
The numbers refer to the manufacturer's (Ilford) code numbers

The solution is contained in a cuvette usually made of glass or plastic, which may be either cylindrical or rectangular in shape. The internal dimension of the cuvette (known as the pathlength) is usually 1 cm. Plastic cuvettes are of lower quality than those made of glass. They are difficult to clean, and to remove films of precipitated material without scratching the internal surface; they are however cheaper. On the other hand glass cuvettes can be better cleaned for reuse and do not deteriorate if properly handled.

Most photometers are calibrated in absorbance units, usually on a logarithmic scale, with values ranging from zero (where  $T = 100\%$ ) to about 2 units, with a further scale mark for a value of infinity (where  $T = 0$ ). In these cases the absorbance reading should be directly proportional to concentration, and calibration will be linear. Sometimes the meter is also calibrated in % T (usually with linear gradations from 0-100), and if this scale is used the calibration curve will be logarithmic. It is usually best to ignore the transmittance scale and use only absorbance readings.

#### Deviation from Beer Lambert law

The analytical procedure is greatly simplified if the relationship of concentration to absorbance is strictly linear. However, the calibration curve **MUST NEVER** be assumed to be linear as it may be influenced by several factors:

- (a) Calibration is more likely to be linear with monochromatic light (i.e. light of a single wavelength). Linearity tends to decrease as the band width of light increases.
- (b) The response of the photocell may not be linear at all wavelengths.
- (c) Some coloured solutions may give a linear calibration on one instrument but not on another. Linearity and other aspects of performance must therefore be judged for each instrument in the circumstances in which it is used (see below).

## SELECTION OF INSTRUMENTS

There are available a large variety of colorimeters, photometers and spectrophotometers of varying complexity and cost. Selection will depend on the intended use. Spectrophotometers are generally more reliable than filter photometers, and the quality of the latter is largely dictated by the quality of the filters and the narrowness of the bands which are separated.

A medium-priced spectrophotometer is suitable for a laboratory requiring to use the instrument for several different chemistry and haematology tests. A central laboratory may require to record the absorption spectrum, e.g., of haemoglobin in order to check the purity of a haemiglobin preparation or to identify abnormal Hb derivatives. For some kinetic analyses, e.g. enzymes, a continuous recording of the spectrum may be required. On the other hand, it may be convenient for the practice of a laboratory to have a simple photometer dedicated to a particular test, e.g., haemoglobinometry, using a colorimeter with a fixed wavelength of monochromatic light.

It should be remembered that these instruments have both mechanical and optical components which require maintenance, including servicing facilities, at regular intervals. Also, they need a stable and constant electrical supply. Some photometers, on the other hand, function on battery power. These include simple portable haemoglobinometers and even simpler comparators which can be used in daylight. The latter are, however, less reliable and are not relevant in the context of this manual.

## OPERATION OF PHOTOMETERS AND SPECTROPHOTOMETERS

### A. Procedures on installation

1. Unpack the instrument carefully and assemble it according to the manufacturer's instructions. Make sure that the operating manual is supplied.
2. Set the instrument up on a level and stable bench where it will be free from vibration and not in direct sunlight. The laboratory environment should be free of dust and fumes, and smoking must not be allowed.
3. Always handle optical components, such as the lamp, filters, cuvettes or lenses by the base or sides, so that fingerprints or other marks are not made on optical surfaces.
4. Check that filters are clean and not cracked or marked. They should be identified by a manufacturer's number or by the wavelength of maximum transmission. Store spare filters in a dust-free container and protected, to try to ensure that they cannot be broken or scratched.

5. Clean cuvettes by soaking for a few hours in a mild detergent or dilute solution of wetting agent. If necessary clean the inside surfaces of the cuvette with a swab of cotton wool. Rinse with distilled water and invert to dry. Store cuvettes in a dust-free container and try to ensure that they cannot be scratched or broken.
6. The cuvette holder (or cell carriage) may accommodate only one cuvette or it may have spaces for 2-4 cuvettes, which can be positioned, in turn, in the light path by sliding the holder. In this case, ensure that the holder slides smoothly and locates positively and in a reproducible position.
7. If a flow through cuvette is provided it must be located in the cuvette holder in a fixed immovable position and connected to a suitable source of suction and/or drainage.
8. Ensure that all lenses, mirrors and other optical components are free of dust and finger prints. If necessary, clean with tissue or a soft cloth. If any optical unit is sealed, **DO NOT** dismantle it to clean it.
9. Connect the instrument to a suitable stabilized electric power supply, taking great care to make sure that the colour-coded wires are connected by a suitable plug to the correct mains terminals. **DO NOT** switch on the instrument without first ensuring that there is a filter in position: failure to observe this may result in damage to the photocell and meter. **DO NOT** leave the instrument on with the photocell exposed to light for longer than necessary. As a precaution it is advisable to block the light path when the instrument is not in use, e.g., by closing the shutter or inserting the cuvette cover in the holder in place of a cuvette.
10. The optical alignment of the system should now be checked to ensure that the maximum amount of light reaches the photocell after passing through the cuvette.

First, with a water-filled cuvette and a suitable filter in position set the meter to a mid-scale reading (roughly 0.3 absorbance or 50% T). Now make minor movements or manipulations of each optical component in turn (lens, mirrors, filters, cuvette, etc.) to see whether stray light from these affects the reading. If necessary make small adjustments so that the maximum transmission (i.e., minimum absorbance reading) is obtained.



Then check the alignment by observing the absorbance maxima of a known reference solution or filter. A 1% solution of potassium dichromate solution (60 mg/l) in 0.005 mol/l aqueous sulfuric acid has the following specific absorbance values:

Wavelength (nm)	Specific absorbance (1 cm pathlength)
235	124.5 ( $\pm$ 1.6)
257	144.0 ( $\pm$ 1.6)
313	48.6 ( $\pm$ 1.6)
350	106.6 ( $\pm$ 1.6)

A holmium oxide glass filter which can also be used as reference has major peaks at the following wavelengths: 241.5 nm, 279.4 nm, 287.5 nm, 333.7 nm, 360.9 nm, 418.4 nm, 453.2 nm, 536.2 nm and 637.5 nm.

The lamp positioning is an important factor. Usually the lamp is prefocused, but minor adjustments in both horizontal and vertical planes may be possible. To make these adjustment easier, it is possible with some instruments to place a white card immediately in front of the photocell, if necessary, after first removing the cuvette holder. A clear image of the lamp filament can usually be seen on the card. If this image is distorted or not vertical, minor adjustments should be made until the best image is obtained.

When a flow-through cuvette is used its position may require careful adjustment. Once this has been done, it is best to leave the cuvette in situ and not interchange it frequently with other cuvettes.

## **B. Operation procedures**

### **1. Check dark current**

Switch on the instrument and set the meter to read infinity on the absorbance scale (0%T) with the shutter closed or the light path blocked so that no light falls on the photocell. This setting should be checked each time the instrument is switched on.

### **2. Check stability of readings**

With a water-filled cuvette in position, set the absorbance reading to a convenient reading such as 0.100. Repeat this reading at 5 minute intervals until it is steady (i.e. changes by less than 0.005 units in 5 minutes). In routine use, never take readings before this warm up period is complete and readings are stable (usually 5-30 minutes).

A major source of error in photometric measurement is the drift of the zero value during determination of a series of specimens. Since the scale of the extinction is logarithmic, the drift of the zero value will result in a change of the difference between the absorbance of the specimen with a given concentration of an analyte and the zero value, resulting in a false estimation. Therefore the drift should be readjusted after each 5 or 10 measurements of a specimen. The zero value must be corrected by measurement of light absorption with an empty cuvette, or a cuvette filled with distilled water or reagent solution.

### 3. Use of cuvettes

After a cuvette is filled with solution, wipe the outside clean and dry, and check that it contains no bubbles or particles. Handle it by the top or sides and not by the optically-transmitting surfaces. After inserting the cuvette in its holder, check that minor movements do not affect readings.

### 4. Matching of cuvettes

Whenever a new cuvette is introduced (e.g., as a replacement for a broken one), it must be matched with the others in the set. This set usually comprises one cuvette containing water or a reagent blank and one or more others containing solutions of the samples, standards, etc. It is essential that all cuvettes in a set give the same reading when filled with the same solution. This should be checked by filling all cuvettes with a haemoglobinocyanide (HiCN) solution. Set the first cuvette at a convenient absorbance reading (e.g., 0.100). Now read each of the others in turn. Repeat all readings and note any which differ consistently by 0.005 units or more. These differences may be due to several causes:

- (a) Dirt or finger marks on the cuvette. Clean the cuvette (see above, A.5) and repeat the test.
- (b) Scratches or imperfections on the optical surface. With cylindrical-shaped cuvettes, this error can sometimes be overcome by rotating the cuvette slowly in its holder and noting the position (if any) where the readings match. This position should be marked and the cuvette always used with the mark in the correct position.
- (c) Variations in pathlength. This effect is only apparent with light-absorbing solutions and not with water.

Any cuvette which, after cleaning, cannot be matched with others in the set should be discarded.

The use of a single cuvette for repetitive measurement of a series of specimens avoids a "cuvette error". The cuvette is filled with a blank solution according to the type of test, the instrument is set to zero by means of this blank. The blank solution is then discarded and the cuvette is turned upside down and shaken to remove the last drops of the contents prior to filling with the solution which is next to be measured. The level of the solution in the cuvette must be high enough that reflections of light from the surface do not interfere with measurement of absorbance (see below, B.5). Also trapping of air bubbles at the walls of the cuvette must be avoided. They can be removed by slightly tapping the cuvette with the finger.

5. Solution volume required

Check the volume of solution required by adding increasing volumes to a cuvette and noting the amount necessary to produce a constant reading. In routine practice it is advisable to use more than the minimum volume, but it is not usually necessary to fill the cuvette to the top.

6. Carryover

Conventional cuvettes are usually rinsed with the next solution before a reading is made in order to avoid errors due to carryover. With flow-through types, the suction arrangement may not remove all the first solution, and it is then necessary to rinse with the second solution, and flush it out, before taking a reading on a further aliquot. It is advisable to check the efficiency of the suction and rinsing process to ensure that errors due to carryover are avoided.

7. External illumination

With a filled cuvette in position check that the reading is not affected by variations in external illumination (e.g., daylight) from above the instrument. If this occurs, the instrument should either be moved or the external light excluded by closing the lid or blocking off the top of the cuvette holder.

**C. Checking linearity and preparation of calibration curve**

Set up a series of five tubes. Into the tubes pipette the following amounts of HiCN reference preparation:

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

Rinse the pipette well in haemiglobincyanide reagent, and then dispense reagent into the tubes as follows:

	Final concentration		
Tube	1	none	100%
"	2	1.5 ml	75%
"	3	3.0 ml	50%
"	4	1.5 ml	25%
"	5	6 ml (approximately)	0%

Mix the tubes well. Measure the absorbance of solutions 1-4 at 540 nm, against solution 5 as blank and plot the readings on arithmetic graph paper, with absorbance on the vertical axis and concentration on the horizontal axis. If readings are in percentage transmittance, semi-logarithmic paper must be used with transmittance on the vertical axis. All points should fall on a line passing through zero (Fig. 2.3).

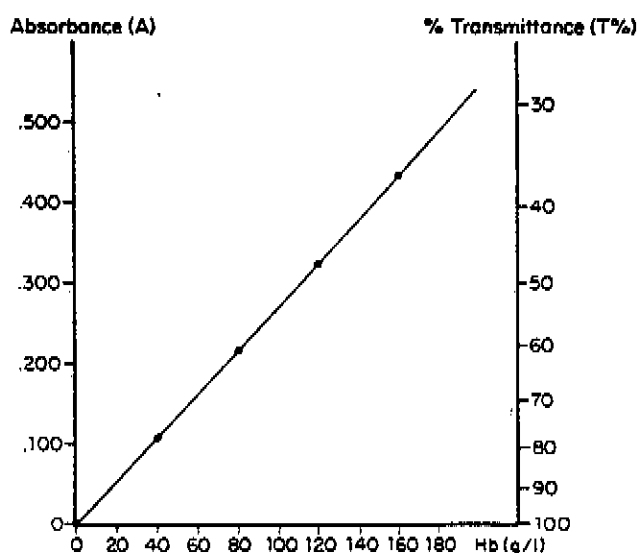


Fig. 2.3  
Calibration graph of haemiglobincyanide

If the calibration curve is linear, perform additional analyses with stronger solutions in order to determine the upper limit of linearity, which ideally should not be less than an absorbance of 1.0.

Suitable solutions for this can be made by converting a sample of whole blood to haemiglobincyanide by means of the HiCN reagent as in the routine procedure for haemoglobinometry, the blood being diluted 1:50 or 1:100 in the reagent to obtain the solution at 100% concentration. Further dilutions of the haemiglobincyanide solutions are then made in HiCN reagent and absorbance is measured as described above.

In routine practice, readings will be reliable only if they lie within the linear part of the calibration curve. With most instruments, the most accurate and precise readings are in the range 0.2-0.7 absorbance units. Readings of less than 0.1 or more than 1.0 absorbance units are less reliable, and critical measurements on patients should, if possible, be made within these limits. If the reading is more than 1.0, or outside the linear range of the method, the analysis should be repeated using a dilution of the sample.

Check the stability of the colour by making repeated readings of one solution for about 30 minutes after preparation. If the colour is unstable, readings must always be made within the time over which the colour intensity is stable.

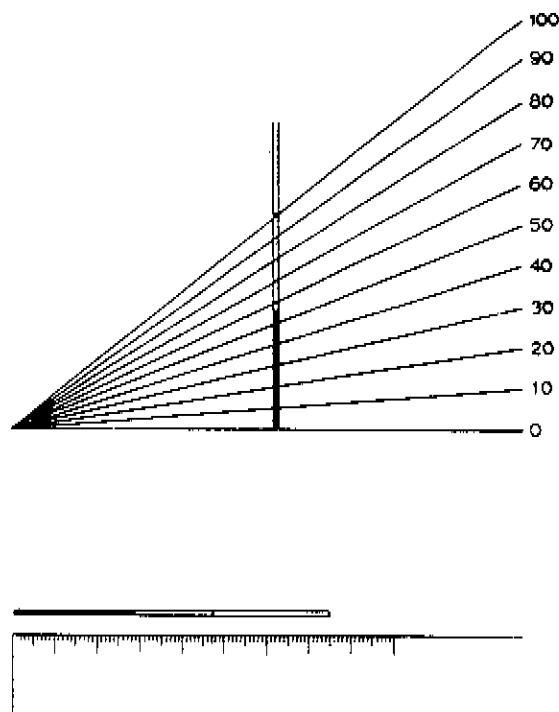
The linearity experiment should be repeated at regular intervals (e.g., monthly) and **ALWAYS** after replacing lamp, photocell or filter.

**PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING** (See Chapter 10)

### CHAPTER 3. MICROHAEMATOCRIT CENTRIFUGE

#### PRINCIPLE

The microhaematocrit centrifuge is intended for centrifuging up to 24 micro-capillary tubes. It should be capable of sustaining a centrifugal force of at least 10 000  $g$  for five minutes. Under these circumstances the red cells become packed at the bottom of the capillary tubes. As the capillary tubes are of uniform bore the erythrocyte volume fraction (packed cell volume or haematocrit) can be estimated by dividing the length of the red cell column by the length of the whole column of blood from the base to the top of plasma. This can be done by placing the tube on arithmetic graph paper and measuring the lengths directly or by using a special reader.



**Fig. 3.1**  
Principle of a packed cell volume reading device

The capillary tubes used are disposable and a regular supply is required. In some developing countries this is not possible and it may be necessary to use Wintrobe tubes and an ordinary bench centrifuge. However the Wintrobe tube requires a larger volume of blood to fill it than does a microhaematocrit capillary; moreover, a much longer centrifugation time is necessary to pack the red cells in the tube.

## SELECTION OF INSTRUMENTS

The instrument must have a built-in timer. Over the period of centrifugation it must sustain a constant  $g$  force of at least 10 000  $g$  without allowing the specimen to overheat and lyse. Safety requirements are of paramount importance; the interior of the centrifuge, the bowl and other parts must be easily cleaned in the event of breakage or leakage from the tubes during centrifugation. Ideally, there should be an automatic locking device to prevent opening of the centrifuge during spinning. In the absence of mains electricity a battery model may be used.

## CALIBRATION

Most laboratories do not have facilities for calibrating the microhaematocrit centrifuge directly. The adequacy of the force is therefore checked by ensuring that it is sufficient to fully pack a specimen in five minutes when  $PCV < 0.5$  and in 10 minutes when  $PCV > 0.5$ . To perform this check select suitable blood specimens anticoagulated in  $K_2EDTA$ , final concentration 1.5 mg/ml in containers which are large enough so that after adding the blood there will be an equal space left to enable proper oxygenation; mix well by inverting at least 20 times and fill 10 capillary tubes. Then centrifuge pairs of tubes for 3, 5, 7, 9, and 11 minutes. There should be no difference between any of the PCV measurements after 5 minutes.

## PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING (See Chapter 10)

## CHAPTER 4. AUTODILUTERS

Autodiluters are also referred to as autopipettes and dispensers. They are used to provide a constant dilution of blood in reagent by a single process.

It is virtually impossible to advise on selection and on maintenance as their designs are so varied. The manufacturer's instructions should be followed carefully. It is, however, necessary to check the calibration of each autodiluter and to make an adjustment to the instrument or provide a correction factor if necessary.

### CALIBRATION

For calibration of the autodiluter, it is first necessary to have a calibrated 0.2 ml (200  $\mu$ l) pipette and 50 ml volumetric flask. Equipment certified and conforming to these specifications is available commercially. The calibration may be checked metrologically by filling the pipette (or flask) to the calibration mark with distilled water which is transferred to a weighed beaker on an analytic balance. The beaker is then reweighed. The ambient temperature is noted. The dispensed volume (in ml) is calculated by dividing the weight of water (in g) by one of the following factors depending on temperature:

<u>Temperature (°C)</u>	<u>Factor</u>
18	0.9986
19	0.9984
20	0.9982
21	0.9980
22	0.9978
23	0.9976
24	0.9973
25	0.9971
26	0.9968
27	0.9965
28	0.9963
29	0.9960
30	0.9957

The calibration must be performed in duplicate for each pipette or flask.

A 2-3 ml specimen of fresh whole blood in EDTA is well mixed, lysed (e.g. by adding a few drops of saponin solution) and then diluted manually 1/251 in haemiglobincyanide reagent using the calibrated pipette and volumetric flask described above.



At the same time a sample of the blood is diluted in haemiglobincyanide reagent (in duplicate) by the autodiluter. Absorbance of each sample is read at 540 nm on a spectrophotometer. The dilution by the autodiluter is obtained from the formula:

$$\frac{A_1 \times \text{Manual dilution (i.e., 251)}}{A_2}$$

where  $A_1$  = Absorbance at 540 nm of manually diluted sample  
where  $A_2$  = Absorbance at 540 nm of autodiluted sample

If indicated, an appropriate adjustment should be made to the autodiluter according to the manufacturer's instructions or a correction factor should be applied whenever the autodiluter is used.

Calibration should be rechecked every three months.

## CHAPTER 5. SEMI-AUTOMATED BLOOD CELL COUNTERS

### PRINCIPLE

Semi-automated counters are the simplest instruments for obtaining blood cell counts; to use them a suitably diluted preparation of blood must be presented to the counter. A known volume is then drawn through the sensing zone of the instrument and counts recorded cell by cell (Fig. 5.1).

Counters use a mercury manometer or other means to ensure that a known fixed volume is drawn through the sensing zone. The process is usually controlled by activation of electrical or optical contacts.

The passage of cells through the sensing zone can be recorded either by the scatter of light or by the change in electrical impedance which occurs. Light scatter instruments are more complex. Aperture-impedance is therefore more suited to semi-automated instruments. With this system there is a change in electrical impedance. The change in impedance is detected and then counted using a suitable electronic circuit (Fig. 5.1).

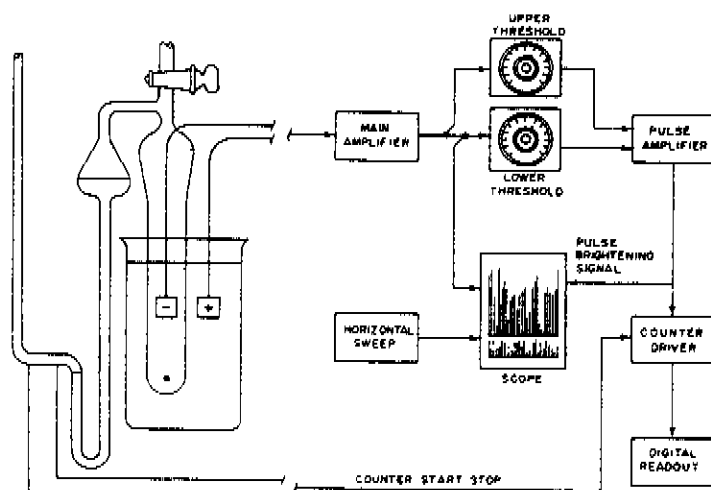


Fig. 5.1

(Reproduced from Richardson Jones, A. (1984), Counting and sizing of blood cells using aperture-impedance systems, in *Advances in Hematological Methods: The Blood Count*, eds Van Assendelft O.W. and England J.M., CRC Press, Inc. Boca Raton, Florida, USA).

A sufficient number of counts must be accumulated within a reasonably short period to achieve adequate precision. The coefficient of variation (CV) of a count,  $n$ , approximates that given by the formula:

$$CV = \frac{100}{\sqrt{n}}$$

For good precision a large number of cells must be counted. In the cell concentration required for this there is the possibility that more than one cell will be present in the sensing zone at the same time. This causes the problem of coincidence loss so that the observed count must be corrected by a coincidence loss correction factor. This varies in different instruments. A suitable correction chart is provided by the manufacturer; in modern counters the correction is made automatically.

Red cells are counted directly on a dilution of whole blood. Many semi-automated instruments also estimate the mean cell volume (MCV) from the average magnitude of the impedance change as the cells pass through the sensing zone. White blood cells are counted after the dilution of whole blood has been exposed to an agent which lyses red cells in such a way that membrane remnants cause signals that are too small to be counted. With some semi-automated instruments platelets can only be counted on dilutions made from platelet rich plasma. The cells being counted are distinguished from debris and other cell types by appropriate threshold settings.

#### **SELECTION OF INSTRUMENT**

As noted above, semi-automated instruments are generally based on the aperture-impedance principle. Unless the instrument is to be operated in an air conditioned environment, it is essential to ensure that the permitted operating ranges are compatible with anticipated ambient temperature and humidity. Transformers, voltage regulators and even filters may be needed to ensure that the electrical supply to the instrument is of the correct voltage; it is essential that the voltage is always maintained at that level and is free from interference. Finally it is necessary to ensure that the effluent from the instrument can be discharged through the laboratory drainage system without causing any environmental hazards, e.g., generation of hydrocyanic acid gas if reagents containing potassium cyanide are allowed to become acidified after discharge; and the risk of explosion if azide-containing reagents are discharged into metallic pipes.

#### **CALIBRATION**

The manufacturer's instructions provided with the counter must be carefully followed in setting it up and using it.

a) Cell count

Some modern counters are preset and cannot be calibrated by the user, i.e., the count measurement process cannot be graduated or adjusted. It is only possible to check the calibration using particle suspensions consisting of preserved blood cells in known concentrations, which may be obtained from the instrument manufacturer or from other suppliers. However, they have only a limited shelf life of approximately four weeks and are expensive to purchase. It may be preferable for the central reference laboratory to make preserved blood preparations with an assigned value in ACD or CPD for distribution to intermediate and district hospitals. Blood that has been stabilized by fixation (e.g., in glutaraldehyde) is also suitable and has a much longer shelf life. It is, however, not suitable for MCV (see below).

b) MCV

Calibration of the counter for MCV is more difficult than for cell count determinations, because there is no predictable relationship between the volume of the red cell and the size of the impulse it produces. Fresh blood can be used for calibrating the instrument, but MCV calibration using preserved blood is complicated by the fact that instruments perceive preserved red cells differently from fresh red cells. This is because the preservation process affects their shape and flexibility and thus the way they are sensed in different aperture-impedance systems. Because of this, commercially available preserved blood calibrants will often have different label values for different instruments. Any central laboratory making such preserved blood cell preparations will also have to take account of this problem, and must assign values to the preserved blood cell preparations from measurements obtained on various types of instruments which have first been calibrated with fresh blood.

Alternatively, if an intermediate or district hospital has validated the accuracy of red cell counting using a suitable stabilized red cell preparation, the MCV calibration can be checked as follows:

Collect fresh blood samples from a number of human subjects whose haematological values are within reference limits. Measure PCV by microhaematocrit and measure MCV and RBC by the counts. If the instrument is correctly calibrated,  $MCV \times RBC$  will agree closely with the PCV.

**PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING (See Chapter 10)**

## CHAPTER 6. SLIDE STAINING INSTRUMENTS

### INTRODUCTION

The preparation and microscopic examination of peripheral blood smears is one of the most frequently performed procedures in diagnostic haematology. It can provide information on blood cell morphology of diagnostic importance. There are however, many pitfalls which may reduce its value and may be misleading. These arise (1) during spreading of the blood smear on a glass slide, (2) because of variation in stain composition, and (3) because of variation in the staining method. Standardization of stain composition and of staining method coupled with meticulous technique will avoid most of these difficulties.

While staining instruments provide a standardized procedure, consistency of composition of stain remains a problem. Most commercial Romanowsky stains contain many impurities which lead to marked variation in observed Romanowsky effect. In addition, it is well-recognized that not only are there marked differences in stain composition from one manufacturer to another, but that similar variation can occur in different batches from the same manufacturer. In an attempt to overcome this problem the International Council for Standardization in Haematology (ICSH, 1984) described a reference method for staining blood and bone marrow films using Azure B and Eosin Y of specified chemical purity to ensure reproducibility of Romanowsky effect. Preparation of stain and the staining technique to be used are also specified in this standard. Adoption of the ICSH standard coupled with the use of a mechanized procedure will, therefore, improve standardization of the overall method. Blood smear spreading also plays a critical part in the process.

Mechanization of the blood smearing process has become an integral component on several commercial instruments concerned with the blood count and automated examination of blood smears. Two methods have been employed: (1) using centrifugal spinners and (2) employing a mechanical wedge smear procedure. Each method has advantages and disadvantages. The mechanical wedge closely duplicates the manual method and produces smears of uniform dimensions, but the distribution error inherent in the method remains. Spinner techniques avoid the distribution error by producing a uniform monolayer of cells over the entire slide. However, variation in sample viscosity can interfere with monolayer preparation and there is potential health hazard because of droplet formation during spinning. The various problems have been largely solved in automated systems in which the spreading unit is an integral component of the instrument. By contrast, simple inexpensive devices which have been produced, both mechanical smear and spinner techniques, are often neither safe nor satisfactory.

### MECHANIZED STAINING DEVICES

These are basically of two types, circular or linear. The earliest form was an adaptation of the conventional histopathology tissue system, in which slide-containing racks are sequentially immersed in a series of stain baths arranged in a ring. For haematology purposes this device is less efficient than the more recently developed linear models. As the former has a capacity far in excess of the

haematology requirements and is wasteful of stain, it will not be discussed further.

### Linear slide staining instrument

The best known instrument of this type is the Hema-Tek slide stainer. The principle of its operation is very simple. The instrument is presented with a previously prepared blood smear. The smear is conveyed, face down, over a precision platen area where metered amounts of stain, buffer and rinse solutions are delivered into the capillary space which exists between the smear and the platen. The platen is a precision component designed specifically to maintain exact volumes of the required solutions within the capillary space and to provide a mixing system for the solutions. A gutter beside the platen allows drainage of used solutions to a waste tank. Three sensing switches are triggered to activate the three solution pumps. After staining and thorough rinsing, the slide is dried by an air-flow from a low velocity blower and the slide is then deposited in a drawer.

### **CALIBRATION PROCEDURE**

The instrument requires no specific calibration. Consistency of staining is assured if the pump volumes are correct since the time phases are constant. A small change in the stain/buffer ratio will produce either lighter or darker staining. While a stain/buffer ratio of 1:2 has been found to give optimal results, this is user-controllable within a very limited range to accommodate individual requirements.

### **OPERATION**

For correct operation the instrument must be on a firm surface and be perfectly level. To achieve this the instrument is fitted with a level gauge inside the hinged lid and two levelling legs located on the bottom of the instrument.

Daily attention is required for:

1. stain tubing and cannula
2. platen
3. waste tank

Careful cleaning of the back and front troughs is required weekly.

The manufacturer recommends only the use of Hema-Tek Stain Pak with the instrument.

**PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING** (see also Chapter 10)

It is usually assumed when staining quality deteriorates that the stain solution is at fault. Frequently other factors are the cause. The following may help in identifying the problem:

1. Improper setting of volume adjustments may lead to poor film quality.
2. Specimen may be old or contain the wrong anticoagulant.
3. Film may be too thick, too thin or unevenly spread.
4. Blood film inadequately dried.
5. Slides not clean.
6. Slides warped.
7. Venting of stain pack containers may be necessary.
8. Inadequate priming of instrument.
9. Stain tubing, cannula, orifices blocked.
10. Dirty platen or groves.
11. Distortion of platen.
12. Defect on conveyer spirals leading to malposition of slide.

**The most common cause of poor staining is a badly prepared smear.**

## CHAPTER 7. ELECTROPHORESIS SYSTEMS

### PRINCIPLE

Electrophoresis is the migration of electrically charged particles in solution or suspension in the presence of an applied electric field. Each particle moves toward the electrode of opposite electrical polarity. For a given set of solution conditions, the velocity with which a particle moves divided by the magnitude of the electric field is a characteristic number called the electrophoretic mobility. The electrophoretic mobility is directly proportional to the magnitude of the charge on the particle, and is inversely proportional to the size of the particle.

In recent years the resolving power of electrophoresis has been greatly improved by introduction of gel supporting media and, more recently, isoelectric focussing.

The gel matrix prevents thermal convection caused by the heat which results from the passage of electric current through the sample. The absence of this convection greatly reduces mixing of the various parts of the sample, and therefore allows better and more stable separation.

In the isoelectric focusing technique, the medium supports a pH gradient which includes the isoelectric pH of the molecule being studied. When introduced into the pH gradient the molecule will migrate to the position of its isoelectric point and then become stationary. This technique is particularly useful for the analysis of protein micro-heterogeneity where only small differences in chemical composition exist.

The major role for electrophoresis in the haematology laboratory is in the identification of abnormal haemoglobins. Cellulose acetate electrophoresis in alkaline buffer (pH 8.5), citrate agar electrophoresis at acid pH (6.0-6.5), and isoelectric focussing, are all satisfactory methods for screening blood samples for sickle cell disease; these techniques also detect beta-thalassaemia major and heterozygous states such as Hb AS, AC and AE. Alpha-thalassaemia-2 trait and beta-thalassaemia-trait cannot yet be reliably detected by these methods.

Cellulose acetate electrophoresis at alkaline pH, followed if necessary by citrate agar electrophoresis at pH 6.0-6.5, are the methods of choice for routine laboratories as they are simple techniques requiring only simple equipment. Isoelectric focussing is an alternative to electrophoresis on cellulose acetate but is technically more demanding and more difficult to interpret. Reagents and equipment are more expensive than those required for cellulose acetate electrophoresis, and electrophoresis on citrate agar is usually necessary.



## SELECTION OF EQUIPMENT

### Power supply

A direct current power supply is required capable of producing 350V and up to 80mA. This is adequate for both methods of electrophoresis but lower voltages and currents may also be used for longer periods.

### Electrophoresis tank

Any horizontal electrophoresis tank which can be set up to bridge a gap of at least 7 cm is satisfactory; however tanks with an adjustable bridge gap are more versatile and permit a variety of different electrophoretic procedures to be carried out. Care must be taken that condensation does not drip on to the cellulose acetate or agar.

### Electrophoretic conditions

- |                       |                  |                                            |
|-----------------------|------------------|--------------------------------------------|
| 1. Cellulose acetate: | Bridge gap       | - 7 cm                                     |
|                       | Voltage/time     | - 350 V for 25 min.                        |
|                       | Current (approx) | - 0.5 mA/cm width gel                      |
| 2. Citrate agar:      | Bridge gap       | - 7 cm                                     |
|                       | Voltage          | - 50 V sponge wicks<br>- 120 V paper wicks |
|                       | Current/time     | - 50 mA for 45-60 min (8x10 cm gel)        |

### Sampler applicator

Suitable applicators can be purchased. Satisfactory results can also be obtained using fine microcapillaries.

## SPECIMEN REQUIRED

While any blood specimen can be used, anticoagulated specimens are preferred. Two to three drops of blood only are adequate for electrophoresis but 0.5-1 ml of anticoagulated cord or capillary blood is useful since it is easier to handle and allows further testing of abnormal specimens. Specimens spotted onto filter paper are also satisfactory if used within one week, but storage in excess of this time results in degradation and interpretative difficulties. Elution from the filter paper is achieved by the haemolysing agent.

### **Haemolysing reagent**

The following are satisfactory:

1. 0.5% v/v Triton X-100 in 100 mg/l potassium cyanide
2. 0.1% w/v tetrasodium EDTA in 100 mg/l potassium cyanide
3. 0.1% w/v saponin in 400 mg/l potassium cyanide.

The EDTA reagent takes a little longer to haemolyse red cells and leaves slightly more stroma at the origin than the Triton X-100 reagent.

### **Haemolysate**

Cellulose acetate electrophoresis: Add one drop of packed red cells to seven drops of haemolysing reagent, mix and allow to stand for five minutes.

Citrate agar electrophoresis: To one drop of packed red cells six drops of haemolysing reagent are added. Some variation in dilution is permissible since the actual dilution required will depend partly on the applicator, partly on the way it is used, and partly on the stain used. Sufficient haemoglobin should be applied to allow minor haemoglobin bands such as Hb Bart's to be detected, but if too much haemoglobin is used there will be poor separation of the Hb A and the Hb F. The technique requires practice and intelligent trial and error.

### **Buffers**

ICSH recommendations for buffer:

#### **Cellulose acetate**

Tris/borate (pH8.5): Tris 10.2g; EDTA (disodium salt) 0.6g; boric acid 3.2g; distilled water to 1 litre.

This buffer may be used up to 10 times without significant deterioration. Some commercial buffers are also satisfactory.

**Citrate agar:** stock buffer: trisodium citrate dihydrate 73.5g; citric acid 3.6g; distilled water to 1 litre.

Working buffer: dilute the stock buffer 1 in 5 with water. Some workers find that this may be used several times without significant deterioration depending on the volume of buffer used in the tank. Some commercial reagents are also satisfactory.

### Reference materials

The ideal reference materials are:

- for cellulose acetate electrophoresis:
  - a) sickle cell trait (Hb A + Hb S + Hb A<sub>2</sub>)
  - b) normal baby (Hb A + Hb F)
- for citrate agar: in addition to a) and b) above
  - c) Hb C trait (Hb A + C)

Alternatively mix normal cord blood with blood from patients with sickle cell trait and HbC trait, thus making a control suitable for major bands such as A, F, S and C.

Satisfactory haemolysates are made by adding packed cells to a detergent or to tetrasodium EDTA. When screening there is no need to wash cells nor to remove the red cell membranes. The addition of cyanide-ferricyanide reagent to haemolysate gives better resolution with older specimens by converting any methaemoglobin present to cyanmethaemoglobin.

Small differences in technique make a major difference to the degree of separation of the haemoglobin bands which in practice can result in failure to detect abnormalities. Two important practical aspects of electrophoretic technique are: (1) the method of sample application and (2) the composition (pH and ionic strength) of the buffer used. When excessive haemoglobin is applied, small bands become obscured when they run near major bands. Optimal separation occurs when a small quantity of haemoglobin is applied lightly in the smallest possible volume to evenly soaked, but well blotted, cellulose acetate. If the surface of the cellulose acetate is too wet when the sample is applied then diffusion occurs. The system should be energized as soon as possible after sample application since delay of even a few minutes can result in diffusion of the sample. Citrate agar gels must be kept cool during the electrophoretic run; otherwise haemoglobin band separation will deteriorate.

It is useful to stain haemoglobin bands since this makes the procedure more sensitive and allows less protein to be applied. Ponceau S has the advantage that it is readily available but it does stain other proteins as well. Alternative stains such as benzidine, o-dianisidine (dimethoxybenzidine) and tetramethylbenzidine are sensitive and specific for haem but they may be difficult to obtain; may fade quickly or are carcinogenic.

**PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING** (See Chapter 10)

## CHAPTER 8. COAGULOMETERS

### PRINCIPLES

These instruments are used to determine the coagulation times in tests such as the prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time or factor assays. Various methods are used to detect clot formation:

#### 1. Mechanical

To detect clot formation mechanically use is made of a metal ball bearing or a stirrer which is caused to rotate in the reaction mixture. Rotation of the ball bearing or stirrer is detected by a simple light system. As the clot begins to form the reaction mixture becomes more viscous and the rotation rate declines. When the time between successive rotations increases over a pre-set threshold coagulation is deemed to have occurred.

#### 2. Photo-optical/turbidometric

In this method increasing optical density (OD) resulting from scattering of light as the specimen clots is detected by a photocell. Differences in OD over successive time intervals are measured ( $\Delta$  OD) and the end point is detected and recorded at the maximum value of  $\Delta$  OD.

#### 3. Nephelometry

Forward light scatter is measured at a pre-determined angle in the reaction mixture. As coagulation takes place the light scatter increases and can be detected by a photocell.

Some instruments may operate by a combination of two or more such methods. The above methods rely on the detection of the fibrin clot but as an alternative it is possible to identify the thrombin released during coagulation. This can be done by using the ability of thrombin to enzymically release a chromogen from an appropriate substrate introduced into the reaction mixture (chromogenic assays).

### SELECTION OF INSTRUMENTS

#### Requirements for use

Before any instrument is obtained it is essential to evaluate whether or not it is required at all and, if so, what facilities should be provided, since coagulometers are available at various levels of sophistication.

The more complex instruments would only be required in centres which are responsible for diagnosing large numbers of patients with clotting disorders such as haemophilia and for monitoring their response to replacement therapy. In practice there are very few such centres and most hospitals, if they require a coagulometer at all, will justify the provision on the basis of their workload of PT determinations. The PT is deranged in liver disease and is one component of the screening tests used to exclude bleeding disorders in patients with a suggestive history who are to have surgery. However, the bulk of the PT determinations are done to monitor the intensity of treatment with oral anticoagulants such as warfarin.

Most hospitals are therefore only able to justify a coagulometer if they have a large workload arising from patients attending anticoagulant clinics. In practice there is probably little point in obtaining a coagulometer unless there are more than 20-30 patients attending any half-day clinic. Once the coagulometer has been provided for such a clinic or clinics it can be used at other times and for other purposes, though more complex determinations which are requested infrequently may still often be best done manually.

#### **Type of specimen**

In general it is more satisfactory to determine the PT on plasma obtained by centrifuging citrated blood specimens. However, some instruments are designed to permit PT determination on citrated whole blood.

#### **Methods of operation**

Semi-automated instruments measure the end point of coagulation but require an operator to be present to apply the sample and reagents. Fully automated systems do not require any operator attention after the samples have been loaded. Such fully automated systems are only required in centres responsible for diagnosing and monitoring replacement treatment in large numbers of patients with clotting disorders.

#### **Detection system**

Because it may be more difficult to obtain reagents for chromogenic assays and because the instruments cannot be manufactured cheaply it is probably better to use systems which rely on the detection of clot formation. Fundamentally there is nothing to choose between mechanical, photo-optical or nephelometric detection and the selection of the instrument is best made with regard to its ability to operate in the likely ambient temperature and humidity. Another very important consideration will be availability and cost of reagents and consumables as well as whether or not a repair service is provided.

## CALIBRATION

The value of the PT cannot be directly used to monitor anticoagulant control since the PT is a function both of the degree of anticoagulation and the thromboplastin which is being used. The following relationships apply:

$$PR = PT \text{ test plasma} / PT \text{ normal plasma pool}$$

$$INR = PR^{ISI}$$

where PR is the prothrombin ratio, INR is the International Normalized Ratio and ISI is the International Sensitivity Index of the thromboplastin. Because the INR adjusts the PT value according to the sensitivity of the thromboplastin it can be used to monitor anticoagulant control. Most modern coagulometers are provided with software to convert the PT to an INR provided that the ISI value for the thromboplastin is fed into the instrument. However, it is absolutely essential to ensure that the manufacturer of the thromboplastin has assigned an ISI value for the coagulometer in use. Unless the coagulometer/thromboplastin combination is correctly calibrated in this way the INR values obtained from the instrument will be inaccurate.

If it is desired to check whether the thromboplastin manufacturer has supplied a correct ISI value for use on the coagulometer the following check should be made: using 20-30 specimens from patients on anticoagulant therapy compare INR values obtained from the instrument with those obtained manually using a thromboplastin whose ISI for manual use is traceable to an International Standard. In the event of discrepancies being found, either the thromboplastin should not be used on the instrument or the laboratory should assign a corrected ISI value for use on the instrument (for more details see: Requirements for thromboplastins and plasma used to control oral anticoagulant therapy. WHO Expert Committee on Biological Standardization. Technical Report Series, 687).

**PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING** (See Chapter 10)

## CHAPTER 9. HOSPITAL BLOOD TRANSFUSION LABORATORY EQUIPMENT

### CENTRIFUGES

#### Centrifuges for general use

These are fitted with swingout or fixed-angle heads. They vary in size and capacity and are used mainly to separate cells and serum or plasma. Some centrifuges may have a refrigeration unit attached.

The centrifuge must be placed on a level surface. All buckets, trunnion rings and inserts must be paired by weight; the weight is marked on all the items. The pairs are weighed to ensure exact matching and are then ready for use. If the pairing is correct no vibration due to imbalance should occur. Centrifuges should not be placed near the edges of benches, or close to balances or microscopes. The normal vibration will cause problems with other instruments.

As the temperature of material being centrifuged will rise in an unrefrigerated machine it is essential to keep spinning time and speed to the minimum for the desired effect.

#### Centrifuges used for serological testing

Two types of small centrifuges are in common use in blood transfusion laboratories. One has a fixed speed and variable time, the other has a variable speed and time.

#### CALIBRATION

A weak antibody should be selected as a standard and stored in small aliquots. Determine the minimum time and speed of the centrifuge that will give a 1+ macroscopic reading with appropriate cells and in addition fulfils the following criteria:

1. Firm agglutination - not easily dispersed
2. Clear supernatant
3. Well defined red cell button
4. Red cell button resuspended without violent agitation.

A negative control should also be included and should show clear negative reactions; e.g.:

positive control: anti-A and A<sub>2</sub> cells  
negative control: anti-A and O cells

The standard should be used on a regular basis to check the performance of the machine. If the expected results are not obtained this may indicate a problem with the centrifuge.

In tests involving media other than saline, i.e., enzyme or albumin, the speed and time may require

slight adjustment. A weak antibody should be tested against positive and negative controls by each technique and the appropriate time and speed chosen to fit standard criteria.

**Example:**

**Albumin technique**

positive control: weak anti-D and D positive cells  
negative control: weak anti-D and D negative cells

**Enzyme technique**

positive control: weak anti-D and D positive enzyme treated cells  
negative control: weak anti-D and D negative enzyme treated cells

**Anti-human globulin test**

1. **Cell washing.** A weak anti-D which gives a 1+ macroscopic reaction with D positive cells in this test is chosen for calibration.

positive control: anti-D and D positive cells  
negative control: anti-D and D negative cells

A set of identical tests should be washed at various times between 30-60 seconds. The time chosen for centrifugation is that which results in a clear delineated button of cells with no cells on the side of the tube.

2. Washed cells + **anti-human globulin reagent.** The centrifugation time required is that which satisfies all the criteria indicated above. This is illustrated in the table below.

Requirements	Time in seconds (fixed speed)					
	10	15	20	30	45	60
Supernatant clear	no	no	yes	yes	yes	yes
Cell button clearly defined	no	no	no	yes	yes	yes
Cell button easily resuspended	yes	yes	yes	yes	yes	yes
Good firm agglutination	weak	weak	weak	+	+	+

**In the above example, the shortest centrifugation time fulfilling all criteria is 30 seconds.** Correct controls must be used at end of test, i.e., cells known to be coated with IgG antibody must be added to all negative AHG test results to prove presence of active anti-human globulin reagent.



## SELECTION

1. Simple construction with sturdy durable casing.
2. Adequate for tasks required and easy to maintain by laboratory workers.
3. Easy availability and speedy delivery of replacement parts.
4. Possibility of use with battery power.

## PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING (See Chapter 10)

### Cell washer for use manually or automatically

## PRINCIPLE

This is a laboratory bench centrifuge capable of washing red cells. Variable spin time settings allow for different uses of the machine. The washer can be programmed to automatically perform 1, 2, 3 or 4 washes per test sequence. Each cycle consists of a defined volume of saline being introduced and subsequent decanting during the centrifugal rotation.

## CALIBRATION

All calibration parameters will have been preset at the factory to accepted values. The operator should be able to use the machine on delivery.

- a) Rotor speed check  
washing cycle and centrifugal rotation every six months
- b) The efficiency of the machine can be assessed weekly by carrying out the antiglobulin test by replicate testing. This should involve all tube positions, and includes the use of standard concentrations of anti-D, four washes and standard reading techniques.

## SELECTION

1. Simple construction capable of performing the specific task.
2. Should work manually and automatically.
3. Simple to maintain by workers in laboratory.
4. Easy availability and prompt delivery of replacement parts.
5. Well tested and tried internationally.

## PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING (See Chapter 10)

### Production of platelet rich plasma

## CALIBRATION

The ideal speed and duration of centrifugation for obtaining platelet rich plasma from whole blood is obtained by experiments in which these are varied and the percentage yield is calculated.

$$\text{Percentage yield} = \frac{\text{Number of platelets in PRP} \times 100}{\text{Number of platelets in WB}}$$

(PRP = platelet-rich plasma; WB = whole blood)

Select the shortest time and lowest speed that produce the highest yield of platelets.

Similarly, the ideal speed and duration of centrifugation for preparing platelet concentrates (PC) from platelet rich plasma can be determined by noting their effects on percentage yield.

$$\text{Percentage yield} = \frac{\text{Number of platelets in PC} \times 100}{\text{Number of platelets in PRP}}$$

The Table below provides an example of the required conditions.

Type of product	Angle head	Swing out head
Platelet rich plasma	4170g 4500revs/min 2 min	1740g 2500revs/min 3 min
Platelet concentrate	5140g 5000revs/min 5 min	5000g 4000revs/min 5 min

## REFRIGERATORS

### PRINCIPLE

Refrigerators provide storage facilities for easily degradable reagents and specimens and products for infusion. Smaller models may work on the absorption principle and can use variable energy sources, e.g., gas, electricity, paraffin, solar power. Larger systems require mains supply electricity and work on a compression system.

#### General points in caring for all refrigerators:

Keep the refrigerator level and allow enough space behind it and between it and other equipment so that air can circulate freely. Refrigerators generate a great deal of heat and if this cannot escape the system will break down.

Always ensure that door is firmly closed after use. The door seal must fit perfectly to prevent warm air from outside entering the cool chamber.

### SELECTION

Refrigerators must be the appropriate size for the job, in order to avoid waste of space and energy, and must be easily cleaned.

For storage of most reagents and samples a domestic refrigerator is suitable. The temperature should be approximately 4 °C but 2 - 10 °C is acceptable. A specially designed refrigerator is necessary for flammable liquids such as ether (see page 66).

The following applies to refrigerators for storage of blood and blood products:

The temperature should be maintained between 2° C and 10° C (preferably between 2° C and 6° C). It should be possible to maintain this temperature for several hours if the electricity supply fails, provided that the door remains closed, and there must be a method for measuring the temperature within the chamber: the minimum requirement is a minimum and maximum thermometer. The refrigerator should contain only blood and/or blood products, and crossmatched and uncrossmatched blood should be in separate areas - preferably in separate refrigerators. It should not contain a freezer compartment.

Ideally, such refrigerators should have inner doors to prevent the entry of warm air, a chart on a seven-day clock for recording the temperature, and visual and audible alarms. The latter should be positioned both in the laboratory and in a remote (central) area. It must be remembered that inappropriate storage of blood and blood products may cause a reduction in their effectiveness or, because of contamination, may be harmful to patients.

### ***NOTHING ELSE MUST BE STORED WITH BLOOD***

**PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING** (See Chapter 10)

### **FREEZERS**

#### **PRINCIPLE**

Freezers provide storage for material that will deteriorate rapidly if left in a normal refrigerator, i.e., patients' sera and blood components such as fresh frozen plasma and cryoprecipitate. They come in a variety of sizes and types and require an electricity supply.

#### **SELECTION**

Domestic type freezers reaching a temperature of - 20 °C are adequate for short term storage of patients' serum samples and most reagents, but are not ideal for long-term storage of serum samples and blood components. Special low temperature freezers (-30 ° C or below) are preferable, but are much more expensive. Freezers should be kept in well ventilated areas. The doors are opened as little as possible, because continual entry of warm air will encourage a build up of ice which will reduce the efficiency of the equipment and put an additional strain on the motor.

Regular defrosting should be carried out as any build up of ice round the door will cause the seal to perish.

An upright or top loading freezer at  $-20^{\circ}\text{C}$  is suitable for general use. It is helpful to have a plan of the location of items in order to avoid opening the door for long periods. All items in the freezer must be labelled.

Freezers at  $-30^{\circ}\text{C}$  or below for storing blood products should be upright with inner doors for each shelf to reduce the intake of warm air when the main door is opened.

An alarm should be fitted to alert staff of change in temperature. The freezer should be capable of maintaining its temperature for several hours in case of electrical failure, provided the doors are kept closed.

**PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING** (See Chapter 10)

## **HEATING SYSTEMS**

### **Water baths**

Water baths serve many purposes in a haematology or blood transfusion laboratory. A very simple type may be used for thawing serum samples, but to carry out coagulation or serological tests requires a constant temperature within a very narrow range.

Water baths used for thawing frozen blood products should not be used for any other purpose.

### **CALIBRATION**

The water bath should be placed on a level surface, filled with water to the required level and covered with a lid to avoid excessive evaporation.

Set the temperature control and allow a period of time for the water to reach the correct temperature. When the required temperature has been reached, check the temperature of the water in various parts of the bath to see if there are any hot or cold areas. If there is a difference in temperature this may be solved by using a continual mixer in the bath. Maintain the water level to avoid overheating and burning out the motor. Replace the water regularly after cleaning and disinfecting the bath.

### **SELECTION**

The bath must be able to maintain a narrow temperature range at the required setting. It should be able to hold a large number of racks and to be easily cleaned, and it should be possible to remove the water easily without lifting it.

**PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING** (see Chapter 10)

### Heat blocks

#### **PRINCIPLE**

Heat blocks can be used as an alternative to a water bath for incubating tubes. They have advantages of size, lack of water and flexibility of movement to different parts of the laboratory.

#### **SELECTION**

Choose the simplest design of an instrument which is easy to clean and maintains the temperature within a narrow range in individuals wells.

**PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING** (see Chapter 10)

### Incubators

#### **PRINCIPLE**

An incubator is a closed chamber designed to provide a constant temperature over a long period of time. The atmospheric pressure, composition of gases and the humidity may be varied, but for blood banking purposes only a constant temperature is required.

The incubator can be used instead of a water bath or heating block and is particularly useful for the incubation of micro-titre plates; the temperature required for this is 37 °C. The inside of the incubator should be easy to clean and the shelves should not tilt during loading and unloading.

It must be on a level surface, and the doors must close very tightly. It must be kept away from heat sources, and moisture at floor level must be avoided.

**PREVENTIVE MAINTENANCE AND TROUBLE SHOOTING** (see Chapter 10)

## **PART II: CARE OF EQUIPMENT**

### **CHAPTER 10. PREVENTIVE MAINTENANCE AND SIMPLE TROUBLE SHOOTING**

#### **INTRODUCTION**

The Manufacturer's Instruction Manual should be read carefully to ensure that the operator knows how to use the instrument. The Manual will also contain instructions on how to keep the instrument in good working order, e.g., by cleaning various parts. Such steps comprise preventive maintenance, i.e., they are maintenance steps intended to prevent the instrument from breaking down.

This Chapter will highlight the key aspects to be considered to ensure that equipment is well maintained and simple trouble shooting will also be described. Certain aspects are general to all or most types of equipment and these will be considered first. Then the aspects which are specific to different types of instrument will be considered in detail.

#### **GENERAL ASPECTS**

##### **Preventive maintenance**

1. Read about operating and maintenance requirements in Manufacturer's Instruction Manual.
2. Adhere strictly to safety procedures (Chapter 11).
3. Keep records of all repairs, maintenance and calibration procedures.
4. Keep instrument clean, i.e., free from contamination with blood, other specimens, reagents and dust.
5. Switch off at mains after use except where the manufacturer advises that the instrument is kept switched on at all times.
6. After use cover instrument with dust cover, if applicable.

##### **Trouble shooting**

1. If equipment operated by mains electricity does not function at all, e.g., no indicator lights come on, check:
  - a) Is it plugged in?
  - b) Check power at plug using test lamp and, if power is off, check any breaker box.

- c) If power is on, check any fuse, either in the plug or the instrument, using a multimeter.
2. If equipment operated by battery does not function, check the charge status of the battery and replace if necessary.

## **MICROSCOPES**

### **Preventive maintenance**

1. The procedures which are described in Chapter 1 should be carefully followed on every occasion that the microscope is used.
2. Every 12 months the microscope should be taken to pieces, cleaned and reassembled by an expert. It is especially important that the specialist inspects the surfaces of the lenses and the prism for the first signs of fungus and takes preventive measures and lubricates the metal parts with a special liquid oil that has cleaning properties.

### **Trouble shooting**

1. If the bulb will not illuminate follow the general trouble shooting instructions (see above). If the electrical supply is adequate try a new bulb: always keep a spare bulb of the correct type and voltage recommended by the manufacturer.

When a bulb is changed, avoid contact with bare fingers as fingerprints reduce light intensity. The bulb can be easily changed by following the manufacturer's instructions. After fitting the new bulb it must be centered again as described in Chapter 1. Life span of bulbs is extended considerably by adjusting voltage to give the lowest required light intensity.

2. To correct other common faults in microscopy:
  - a) Is the illumination correctly set up? (See Chapter 1)
  - b) Does the image lack contrast? If so clean objective lens, check for damage to objective.
  - c) Unsharp patches in microscope image are usually caused by dust on lenses or optical surfaces. Locate by rotating or moving in turn eyepiece, objectives and mirror, etc.
  - d) Does the image lack contrast with oil objective? If so, has oil been omitted? Check front lens for old residual oil; check if oil contains bubbles.
  - e) Does the image lack contrast with large aperture dry objectives? If so, has the cover glass been omitted or is it too thick or too thin? Is there too much mounting medium?



- f) If the specimen cannot be focused, has the microscope slide been placed with coverglass facing downwards or coverglass mounted on wrong side?
- g) If the image is unsharp after an objective change, check that the objective has been screwed fully into revolving nose piece.

## PHOTOMETERS AND SPECTROPHOTOMETERS

### Preventive maintenance

1. Always follow the manufacturer's recommended maintenance schedule. Keep records of reading of standards and reagent blanks. This may be a useful indication of slowly deteriorating performance. Records should also be kept of the date when any parts were replaced, or other adjustments made.
2. Always clean the instrument after use, paying particular attention to any spillages within or on the surface of the instrument. When the instrument is not in use, turn it off, allow it to cool and cover it to exclude dust.
3. Do not leave dirty cuvettes in the instrument after use. Wash them with distilled water and allow to drain before storing them in a dust-free container. If cuvettes become greasy or contaminated with protein after prolonged use, soak them overnight in a detergent solution. If a deposit remains, use a solution made from equal parts by volume of ethanol and 3 ml/l HCl. Never use any brush or instrument that might scratch cells. Do not use strong alkalis or chromic acid. Store in a dust-free container to ensure that they cannot be scratched.
4. Do not allow filters to become hot by, for example, leaving them exposed to the lamp for long periods. Inspect them regularly for clarity and if necessary clean them with a soft cloth to remove dust. Always leave a filter in position so that the photocell is not damaged when the lamp is turned on. Store spare filters in a dust-free container.
5. Turn off the lamp after use, as it has a finite life. Do not place objects on top of the colorimeter as these may impede air flow and cause overheating.
6. At regular intervals, clean the other optical components on which dust may have accumulated. If any adjustments are made, check the optical alignment as described in Chapter 2 A.10 (p.16).
7. The front surface or window of the photocell should be inspected periodically in case dust or chemical deposits have accumulated. Clean the surface with a soft cloth. Do not dismantle the photocell housing unless the photocell is to be replaced.

### **Repairs and replacements**

1. As a minimum it is recommended that the laboratory should keep several spare lamps, fuses and cuvettes. Other spare parts to be kept depend on the type of instrument and their availability locally when required. Photocells will deteriorate if stored in hot or humid conditions.
2. Keep a supply of spare **fuses** of the type specified by the manufacturer. Replace when necessary.
3. **Lamps** slowly deteriorate during use and the glass envelope darkens, resulting in loss of light output and poor performance. The useful life of the tungsten lamp is estimated at 12000 hours when operated at a potential of 5.4 volts with a current of 4.5 amperes. The life of the lamp is markedly decreased by an increase in the operating voltage. The end of the lamp's operating life is denoted by failure to start or by rapidly decreasing energy output. Replace such lamps before they fail completely, using the type supplied or recommended by the manufacturer. If this is not available, great care is needed in selecting a suitable alternative. After replacing the lamp, check its alignment (p.16) and the linearity of the calibration curve (Chapter 2 C, p.19).
4. Scratched or damaged **cuvettes** should be replaced immediately by others of the same type from the same manufacturer. Conventional test tubes are not usually a satisfactory substitute for cylindrical cuvettes, as they are rarely optically matched. If cuvettes from another manufacturer must be used, it is best to keep these as a separate set, as different types may not be optically matched. Any new cuvette must be optically matched with others in the set (see Chapter 2 B4, p.19).
5. Replacement of the **photocell** is necessary only occasionally. The type recommended by the manufacturer should be used, and his instructions followed. After replacing of the photocell, check the optical alignment (see Chapter 2 A.10, p.16) and the linearity of the calibration curve (see Chapter 2 C, p.18).

### **Trouble shooting**

1. **No reading with water blank**
  - a) Check that the lamp is on. If not:
    - check switches and terminals
    - check lamp positioning in socket
    - replace fuse
    - replace lamp

b) If the lamp is illuminated:

- check whether the meter is locked or stuck
- check for loose photocell connections
- meter failure is unlikely, but if everything else fails, replace it.

**2. Drift in water blank reading**

a) Insufficient warm-up time

b) Lamp failing or overheating:

- Inspect and if necessary replace

c) Failing photocell (e.g., overheating or overexposure)

- Switch off and allow the photocell to rest. If the problem persists, replace the photocell.

**3. Water blank reading fluctuates and cannot be set to zero**

a) Mains voltage variations

- Examine light intensity

b) Faulty potentiometer

- Rotate the knob slowly. If the response is erratic, the slide wire may be dirty or worn unevenly. Clean the slide wire carefully. If the problem persists or recurs consider replacing the potentiometer according to the manufacturer's instructions.

**4. Reagent blank reads less than water blank**

a) Cuvettes incorrectly positioned:

- Try interchanging them

b) Dirty water blank cuvette:

- Clean it

c) Check solutions for turbidity

**5. Daily standard reading variable**

- a) Fault in method or technique
- b) Incorrect, dirty or misaligned filter
  - Check and clean it, if necessary
- c) Positioning of cuvettes variable:
  - Check movement of cuvette holder
- d) Dirty or mismatched cuvettes:
  - Inspect and clean if necessary
  - Check matching
- e) Change in linearity:
  - Check (see Chapter 2 C, p.19). For possible causes see below.
- f) If the lamp or photocell is failing, deterioration is likely to be progressive and there should be other signs of failure. If necessary, replace.

**6. A previously linear calibration curve becomes non-linear**

- a) Fault in method, reagents or technique
- b) Misaligned optics
  - Check and, if necessary, adjust
- c) Stray light due to dust accumulation on the optical system
  - Clean and adjust
- d) Deterioration of filter
  - Inspect and, if necessary, clean or replace

- e) Lamp failing
  - Inspect and, if necessary, replace
- f) Photocell failing
  - Check and, if necessary, replace

## **MICROHAEMATOCRIT CENTRIFUGES**

### **Preventive maintenance**

1. The microhaematocrit centrifuge should be checked for blood spillage daily or more frequently if heavily used.
2. If there has been breakage or leakage from the tubes during centrifugation wear gloves to clean the instrument. Glass pieces should be removed with forceps and the rotor cleaned with gluteraldehyde on a cotton wool swab (see Chapter 11).

### **Trouble shooting**

1. If the centrifuge will not operate, follow the general trouble shooting instructions (p. 47). If the electrical supply is adequate unplug the centrifuge and look for obvious problems:
  - a) A blackened spot may indicate a short circuit.
  - b) A loose wire may indicate a broken lead.
  - c) Melted resin, smoke deposit or strong smell may indicate a burnt out transformer or motor.
  - d) Look for split resistors.
2. If motor not functioning or intermittent:
  - a) Check power to motor.
  - b) Can the motor be turned by hand? If it can, unplug the device and check brushes. These are usually accessible on either side of the motor. Check amount of carbon on brushes. Is the brush touching the armature? Check that the spring wire is not broken. Replace with brush of correct size after removing carbon build-up in brush holder or motor with solvent (carbon tetrachloride).
3. If motor will not turn, bearing can be oiled with light oil. If unsuccessful, bearing probably worn and must be replaced.
4. If glass capillary tubes are constantly breaking, check rim gasket for wear; replace if necessary.

## **SEMI-AUTOMATED BLOOD CELL COUNTERS**

### **Preventive maintenance**

The orifice tube is the most important part of the sensor in aperture-impedance systems and the flow cell is the most important part in light-scatter systems.

#### **1. Sensing zone**

- a) During regular use the instrument sensor, either orifice tube or flow cell, may become blocked by debris or over-sized particles. This will restrict the flow through the sensing zone with a resulting erroneous count. Blockages like this require cleaning.
- b) Temporary blockages may be cleared by flushing the orifice or flow cell with diluent or gently agitating with a fine brush.
- c) Permanent blockages require the removal of the orifice tube or the flow cell from the instrument and backflush of diluent under gentle pressure will be required.
- d) For stubborn protein deposits other cleaning agents may be required, with the orifice or flow cell soaking submerged in the solution (e.g., 50% nitric acid). Manufacturer's recommendations for choice of cleansing agent should be sought at all times.
- e) Low power ultrasonic baths may be used, particularly where flow passages are difficult to reach, as in flow cells.

#### **2. Manometer**

Some older semi-automatic blood counters use a mercury manometer, by which the counting circuit is turned on and off. The liquid in contact with the mercury surface can become extremely dirty. Also the mercury itself can become "sticky" and need replacing. (Mercury cleaning is not recommended other than by an experienced service engineer).

The manometer itself should be cleaned every 3-4 months depending upon the use of the instrument. Instructions for this can be found in manufacturer's recommended maintenance procedure.

#### **3. Tubing**

Leaks in the tubing may cause air bubbles to form which affect the count.

#### 4. Vacuum system

Most instruments today vent directly into drains; however, smaller instruments have waste-trap bottles.

- a) Regular emptying of any waste bottle is required.
- b) Regular inspection of seals is required to determine the possibility of vacuum loss.
- c) A disinfectant should be placed in the clean waste-trap to neutralize any bacterial growth in the effluent.
- d) A de-foamer can also be added to the waste-trap to stop any liquid getting into vacuum pumps etc.
- e) All liquid emptied from the waste-trap is potentially hazardous and should be treated as such (see Chapter 11).

#### 5. General decontamination procedures

All manufacturers provide a recommended decontamination cleaning protocol for their instruments. This must be carried out at the end of a shift or working day, and also before maintenance work on the internal parts of the system is undertaken:

- a) A suitable strength hypochlorite solution is aspirated throughout the instrument, via both sample and reagent lines.
- b) After a period of time, distilled water is flushed through the instrument before resuming normal working.

#### Trouble shooting

Trouble shooting can vary considerably from instrument to instrument. However, there are a number of basic points which should be considered in the first instance.

1. If the instrument will not operate, follow the general guidance for electrical equipment (p. 47).
2. If there is electrical interference this may result in:
  - a) Internal noise or interference which can be detected by some impedance type analyzers. Often by switching off aperture current, the pattern on the oscilloscope screen may indicate the fault, e.g., improper grounding or cracked manometer tube.

- b) External noise: shielding provided by the instrument's cabinet may be faulty and therefore external noise may be picked up. A mains filter may be required to filter out interference from the mains supply.
  - c) Signal noise from an inappropriate diluent.
  - d) Electronic component failure; this requires a service engineer to repair the instrument.
3. If the instrument fails to aspirate the sample, check for blockages, leaks or lack of vacuum.
  4. If the instrument aspirates but gives an aberrant count, check for blockages in the orifice or flow cell, incorrect threshold settings, faulty electrical connections, e.g., leading to absent aperture current.
  5. If the vacuum is absent, look for leaks in vacuum lines and the manometer. The only other possibility is that the vacuum pump is not functioning correctly.
  6. If the counts are showing excessive variation or are erratic, check:
    - a) sample preparation and mixing
    - b) whether the correct diluting fluid has been used
    - c) the glassware
    - d) the settings and calibration

**Other sources of error even if the counter is functioning correctly**

1. The diluent may have a high background count or is otherwise unsuitable for the instrument.
2. The dilutions are inaccurate.
3. The dilutions contain air bubbles because of vigorous shaking or dispensing.
4. The threshold settings may be wrong.
5. The aperture current may be too high and cause damage to the cells.
6. Haemolysis or volume change of red cells may occur if there are residual traces of lytic agent or detergent cleaner in the dilution containers if they are reused.



## **ELECTROPHORESIS SYSTEMS**

### **Preventive maintenance**

1. Ensure that all electrical terminals are clearly labelled and color coded in accordance with a national electrical convention.
2. Ensure that wiring is properly insulated and free from damage.
3. The terminals should be kept dry and only the electrodes should come into contact with the buffers used to perform the electrophoresis procedures.
4. Keep condensation in the tanks to a minimum. If necessary switch the equipment off and remove excess condensation before continuing the run.
5. Ensure that any safety features are operating correctly, e.g., the circuit is broken if the lid is removed.
6. Systems with a cooling platen must only be used if there is a constant supply of cool running water. The tubes which carry the water supply must be bleached at intervals to prevent growth of algae.
7. Keep gel plates moist prior to use so that they do not dry out.

### **Trouble shooting**

1. If the electrophoresis system will not operate follow the general trouble shooting instructions (p. 47). If the electrical supply is adequate switch the equipment off and check:
  - a) The electrodes and terminals are connected by good contacts.
  - b) No wires are broken.
  - c) The correct volume of buffer is present to allow electrical contact.
  - d) The wicks are moist and in contact with the buffer and the supporting medium.
2. If the resolution is poor it may be that:
  - a) The haemolysate was too concentrated.
  - b) The run was interrupted by an unstable or intermittent power supply or the run was for too short a period.
  - c) The buffer pH is wrong.
  - d) The gel plates are dry or torn.
  - e) The applicator was dirty.
3. If the gels become cracked or burnt it may be that:

- a) The contacts were poor.
  - b) There has been excess condensation.
  - c) The gel plates were dry before use.
  - d) The cooling supply (if any) was inadequate.
4. If the samples run in the wrong direction this may be because:
- a) The samples were applied at the wrong end of the gel,
  - b) The connectors were the wrong way around.

## **COAGULOMETERS**

### **Preventive maintenance**

The procedure to be followed will depend on the complexity of the instrument and the type of detection unit.

1. Perform the following checks daily:
  - a) Examine reagent tubing
  - b) Rinse tubing with distilled water at the end of each day and when the reagents are changed
  - c) If there is a peristaltic pump ensure contacts with tubing are lubricated as recommended by the manufacturer
  - d) Ensure that pump volumes are correct
  - e) Ensure that the temperature is correct both daily and between batches. The temperature may be displayed or read on a special thermometer.
2. Perform the following checks monthly:
  - a) If necessary replace tubing, filters, etc.
  - b) Clean the pumps, ensure they rotate freely and lubricate as recommended by the manufacturer. Then check that pump volumes are correct.
3. If the instrument has an inbuilt diagnostic programme it may be possible to check various electronic functions.

### **Trouble shooting**

1. If the instrument will not operate, follow the general guidance for electrical equipment (p. 47).
2. When excessive variation or erratic results are obtained check the following:
  - a) The controls and reagents are reconstituted with the correct volumes of the correct diluent, have

- been stored correctly and are properly mixed.
- b) The incubation and activation times are correct
- c) The pump volumes are correct
- d) The samples have been properly collected and handled
- e) The delivery systems are clean, no tubing is twisted and the reagent dispensing tips are not blocked.
- f) The temperatures are correct.

## **CENTRIFUGES - BENCH AND REFRIGERATED**

### **Preventive maintenance**

1. Perform the following checks daily:
  - a) Clean buckets and inserts, invert to dry
  - b) Examine interior for spillage and clean as necessary
  - c) Check lid lock
  - d) Check temperature of refrigerated centrifuges
2. Perform the following checks monthly:
  - a) Check plugs and cables for wear
  - b) Check brushes and replace if necessary
  - c) Check trunnion rings and buckets for wear
  - d) Check for corrosion.

### **Trouble shooting**

1. If the centrifuge will not operate, ensure that the lid is correctly closed and the correct speed setting has been chosen. Then follow the general guidance for electrical equipment (p. 47) and trouble shooting as for the microhaematocrit centrifuge (p. 53 items 1 and 2).
2. If there is excessive vibration ensure that:
  - a) The trunnion rings and buckets are undamaged and seated correctly
  - b) The tubes are balanced (check by weight).

## **CELL WASHERS**

### **Preventive maintenance**

1. Perform the following daily:
  - a) Clean centrifuge, and remove any build-up of saline
  - b) Check lid interlock
  - c) Check adequate levels of saline in reservoir
  - d) Check saline tube into machine
  - e) Check waste line from machine
  
2. Perform the following every month:
  - a) Empty, clean and refill saline reservoir
  - b) Check tubing for signs of wear and replace as necessary.

### **Trouble shooting**

1. If the cell washer will not operate, follow the general guidance for electrical equipment (p. 47).
  
2. If there is excessive vibration:
  - a) Check that the carrier is correctly seated
  - b) Ensure that the tubes are balanced properly
  
3. If the incorrect volume of saline is dispensed to the tubes check the following:
  - a) There is sufficient saline in the reservoir
  - b) The saline delivery tube is not broken, bent or blocked
  - c) The setting for delivery is correct
  - d) The correct size of tube is being used.
  
4. If there is incorrect or variable saline residue in tube, ensure that the correct size of tube is being used. If the decant speed is incorrect, it will be necessary to have repairs carried out by the manufacturer's agent.

## **GENERAL PURPOSE REFRIGERATORS**

### **Preventive maintenance**

1. Perform the following daily:
  - a) Check temperature
  - b) Remove any water on the floor of the refrigerator
  - c) Check fuel supply if applicable
2. Perform the following monthly:
  - a) Clean inside
  - b) Defrost if required
  - c) Check door seals

### **Trouble shooting**

1. If the refrigerator will not operate or the temperature fluctuates, follow the general guidance for electrical equipment (p. 47).
2. If the compressor has failed repair or replacement will be necessary.

## **BLOOD BANK REFRIGERATORS**

### **Preventive maintenance**

1. Perform the following daily:
  - a) Check temperature
  - b) Check the chart if a recorder is in use, otherwise read the maximum and minimum temperatures and note them in a record book
  - c) Remove any water on floor of the refrigerator.
2. Perform the following weekly:
  - a) Check that any local and remote alarms are functioning
  - b) Change the chart on the recorder if one is in use
  - c) Check door seals.

### **Trouble shooting**

As for general purpose refrigerators.

If there is interruption in power supply, check reason; if it is likely to continue for more than 1-2 hours, switch to alternative power source or transfer contents to another refrigerator as soon as possible.

Do not open the door of the refrigerator during the period of failure unless absolutely necessary.

## **FREEZERS**

### **Preventive maintenance**

1. Check the temperature daily.
2. Check the door seal weekly and defrost as necessary.

### **Trouble shooting**

1. As for general purpose refrigerators; also, as for blood bank refrigerators if used for storage of blood components.
2. If there is temperature fluctuation, check if there has been a build up of ice causing a break in the door seal; if necessary, defrost and recheck temperature maintenance.

## **WATER BATHS**

1. Check temperature at least daily.
2. Check water level daily and maintain at an adequate level.
3. Empty, wash and refill waterbath every month to prevent growth of algae. Then recheck temperature prior to use.

### **Trouble shooting**

1. If the waterbath will not operate, follow the general guidance for electrical equipment (p. 47).

2. If the waterbath is not maintaining temperature (within  $\pm 0.6$  °C of the desired temperature):
  - a) Is the thermostat incorrectly set? If so adjust temperature setting.
  - b) Is the amount of water inadequate? If so increase level of water.

### **HEAT BLOCKS**

1. Check daily on random wells to test length of time for a standard volume to reach the required temperature.
2. Check plugs and cables monthly and replace as required.

#### **Trouble shooting**

1. If there is total failure follow the general guidance for electrical equipment (p. 47).
2. If replicate tests give inconsistent results, clean walls of block and then test temperature of individual wells.

### **INCUBATORS**

#### **Preventive maintenance**

1. Check temperature in various areas to ensure consistency daily.
2. Check plugs and cables monthly and replace as required.

#### **Trouble shooting**

1. If there is total failure follow the general guidance for electrical equipment (p. 47).
2. If temperature is not maintained, check if thermostat is incorrectly set: if so adjust temperature setting.
3. If the temperature fluctuates:
  - a) Check for damage to thermostat and replace if required
  - b) Check that the insulation is adequate and intact.

## **CHAPTER 11. SAFETY IN THE HAEMATOLOGY LABORATORY**

### **GENERAL PRINCIPLES**

This chapter is intended for use by those working in routine medical laboratories. It provides a specification for staff safety in relation to the use of standard haematology instruments, reagents and supporting equipment. It is not intended as a complete treatise on health and safety in the laboratory and should be read in conjunction with appropriate local and national regulations and guidelines.

While it is accepted that the laboratory director or manager is ultimately responsible for the definition and written description of safety policies and procedures, together with staff education in these matters, it must be stressed that all employees of the laboratory are responsible not only for their own safety but also for that of their colleagues and that of any third parties who may be present in the laboratory. This last includes patients, nurses, hospital tradesmen and porters and also commercial service engineers. Safety rules need to be more stringent and protocols more detailed in circumstances where the involvement of professionally trained staff is minimal or absent. All documents pertaining to safety must be written in a language which is readily understood by employees.

The creation of a safe working environment depends on the adequate initial training of the staff working there. Staff must not be permitted to use laboratory equipment nor perform any laboratory procedures until they have received formal training according to a designated programme and have demonstrated their ability to use equipment and undertake procedures safely. From time to time, retraining and confirmation of competence in the use of equipment and procedures are required.

Safety policy must be described in an overall laboratory Code of Practice. In addition there must be specific protocols detailing general safety procedures, for example, personal hygiene, safe handling of specimens, dealing with laboratory spills and these must be supported by fully documented Standard Operating Procedures (SOPs) with adequate cross reference to analytical method protocols. The latter must always specify relevant safety, decontamination or treatment actions to be taken in the event of accidents. They must, in addition, include details of where to find help or guidance in case of problems. Staff must be required to indicate in writing that they have read and understood these procedures.

### **TRAINING PROGRAMME**

The training must be defined to ensure that all employees receive appropriate safety instruction for the procedures performed in the laboratory. Laboratory management, at the same time, must ensure that adequate facilities exist to provide a safe and healthy working environment in accordance with current legislation or local rules.



**Clothing.** Suitable protective clothing must be worn in laboratory areas and when venesecting patients. When not in use, laboratory coats must be hung on pegs located near the exit and not kept in personal lockers. The latter must be located outside the laboratory area and must be used only for everyday clothing.

**Laundering.** Laboratory coats must be laundered through the hospital service and on no account should be taken home. Laboratory protective clothing should be sent to the laundry in special bags and washed in those bags without being removed. If disposable protective clothing is used this will require to be disposed of along with other contaminated waste. Additional protective clothing, such as disposable gloves, aprons and safety spectacles or visors, must be provided if it is essential to handle high risk materials or hazardous reagents, and when disinfecting surfaces and instruments.

**Hygiene precautions.** Smoking, eating, drinking, sucking sweets or chewing gum, or the application of cosmetics are absolutely prohibited in laboratory areas and passages, or other places where specimens are handled, e.g., phlebotomy areas. Labels must not be licked; hands must be washed after any procedure in which they may have become contaminated with chemicals, blood or other biological materials; fingers must not be used to occlude tubes when the contents are being mixed. Cuts and grazes must be covered by a suitable water-proof dressing.

**Mechanical hazards.** Staff must not wear pendant jewelry in the laboratory area. Ideally watches and rings should also be removed and long hair contained. Personal clothing must not be allowed to protrude beyond the sleeves of protective clothing. Since lifting equipment often causes back injury, instruction in this procedure must be provided.

**Rest room.** A rest room must be provided where staff can eat and drink. Laboratory coats, reagents, blood or other specimens must not be taken into this room. Hands must be washed before entering the rest room.

**Pipetting.** This is a frequent procedure in hospital laboratories. Mouth pipetting must be totally prohibited. Staff therefore need training in the use of bulb pipettes or other hand pipetting devices.

**Waste disposal.** Various categories of clinical waste exist and written procedures must be prepared for the disposal of each. Staff must be trained in these methods. All blood specimen containers, disposable ESR tubes and syringes, and contaminated swabs, should be placed in plastic bags and incinerated. The plastic bags should be sealed and labelled "Infective risk for incineration" before leaving the laboratory. If incineration is not available, bags must be autoclaved before disposal. All sharps, i.e., needles, lancets, capillary tubes and broken glass, should be sealed in a stout labelled container before incineration. At the end of each day 0.5 litres of 2.5% hypochlorite solution should be poured down each sink into which the waste solution from cell washers drain.

**Broken glass.** Broken glassware must not be used. It must not be placed in ordinary bins but disposed of in a separate sealed container which must be clearly labelled. Any contaminated glass should be placed in special bins for disposal by incineration (see above).

**Use of centrifuges** (including cell washers). Centrifugation is performed frequently in the laboratory and new entrants to the laboratory must receive instruction in its correct performance at the earliest opportunity. Centrifuges must have locks to ensure they remain closed during operation. Tubes containing blood or other body fluids must be stoppered before being placed in the centrifuge. The risk of breakage within the centrifuge is minimized by careful balancing. Inbalance will cause the centrifuge to move. Trunnion rings or buckets which are unbalanced or are incorrectly positioned may cause extensive damage and are dangerous to people in the laboratory. The interior of the centrifuge must be cleaned daily with a suitable virucidal disinfectant. Gloves are worn during this procedure. Decontamination procedures in the event of spills are described later.

**Care of work places.** Benches must be wiped with 1% hypochlorite at the end of each working day. Gloves must be worn for this procedure. Accidents are most likely to occur when the work place is cluttered with equipment and materials and therefore care must be taken to ensure that it is tidy at all times. Equipment must be kept clean.

**Wash-up procedure.** Glassware contaminated with blood must be disinfected by soaking in hypochlorite solution before being sent for washing.

**Refrigerators.** It is important to recognize that food and drink may only be kept in cabinets specially reserved and labelled for this purpose and that such must be located in the rest room and never in the laboratory area. It is also important to distinguish the domestic refrigerator from that designed for use in the laboratory. **The normal operation of the electrical circuitry may cause ether and other volatile liquids kept in domestic refrigerators to explode.** A refrigerator specially designed for flammable solvents must be used for storing such materials.

## **SAFETY OFFICER**

Every laboratory must have a safety officer trained in general laboratory safety and in any specific hazards occurring in that laboratory. The safety officer should participate in the general work of the laboratory. The safety officer is appointed by management and is responsible to management. The safety officer must visit all parts of the laboratory at regular intervals to ensure that adequate safety precautions are being maintained. A record of this inspection must be made and forwarded to the head of department together with any recommendations made. All maintenance staff and service engineers must report to the safety officer or senior technician before starting work when they should be informed of any precautions which should be taken.

## REPORTING ACCIDENTS

Accidents and incidents affecting the health of laboratory employees must be recorded in a book kept solely for that purpose. The following information must be recorded:

- 1) Person(s) involved (including witnesses)
- 2) Patient's name if specimen involved
- 3) Nature and site of accident
- 4) Action taken

The relevant information must then be entered in the Hospital Accident Book and a copy taken to the hospital administrator. Following an accident where a member of staff is injured, a senior member of the medical staff should be notified and the injured person must be seen in Casualty or by the Occupational Health Service.

Laboratory management must review the accident book regularly and take whatever action is necessary to prevent recurrence.

## PROVISION OF FIRST AID

There are two requirements. First a number of staff must be trained in first-aid procedures. A list of these individuals must be displayed together with the telephone numbers of the various emergency services. Secondly, a first-aid box must be provided which must be clearly identifiable as such. The first-aid box must be constructed from materials which will keep the contents dust and damp-free. The contents of the first-aid box should be restricted to:

1. Instruction sheet giving general guidance
2. Individually wrapped sterile adhesive dressings in a variety of sizes
3. Sterile eye-pads with attachment bandages
4. Triangular bandages
5. Sterile coverings for serious wounds
6. Safety pins
7. Selection of sterile but unmedicated dressings.

All items must be correctly stored and regularly checked to ensure that they are in satisfactory condition. The contents of the first-aid box must be replenished immediately following use.

Eye-irrigation equipment must also be readily available, either in the form of a wash bottle (the contents of which must be changed regularly), or a system connected to the mains water supply. Where tap water is not available, sterile water or sterile normal saline in disposable containers must be available. The correct use of eye-irrigation methods must be demonstrated.

Antidotes to poisonous chemicals used in the laboratory must be available with protocols for their

use.

Protective clothing and safety equipment must be provided for the person rendering first-aid.

### **FIRE IN THE LABORATORY**

Fire prevention is normally subject to statutory regulations which should be known to all employees. Fire is a particular risk in the laboratory because of the flammable solvents, gases and reactive chemicals located there and the large amount of electrical equipment in use. Many fires result from accidents with flammable solvents or with specimens treated with them. An example of this risk with domestic refrigerators has been described above. As a general principle all manipulations involving the use of flammable solvents must be performed in a fume cupboard to avoid accidental ignition of vapor by naked lights. While day-to-day supplies of flammable liquids can be stored in the laboratory in flameproof cabinets, bulk stocks must be kept in an external "Flammable Store".

Fire detection and alarm systems should be installed in all laboratories and in many countries this is a statutory requirement. Heat detection systems are desirable for laboratory areas whereas smoke detection units are preferred for offices, rest rooms, stores, records rooms and computer suites. If the laboratory is unmanned at any time, fire detection systems must be linked to the hospital telephone switchboard. Breakglass call points (using a small hammer to break the glass) should be installed which are capable of triggering the system. In addition, audible alarms which can be heard throughout the laboratory should also be installed.

Fire extinguishers should be located close to all critical areas including solvent stores and fume cupboards. These must be capable of dealing with both chemical and electrical fires. Dry powder or carbon dioxide fire extinguishers are most appropriate since they are capable of dealing with both types of fire. Fire extinguishers require regular maintenance, details of which are usually incorporated in the labelling on the device.

The following items should also be available for fire fighting to ensure the safety of the fire fighters: sand bucket, asbestos, blanket, goggles and possibly a respirator.

Fire risk and the location of Fire escapes must be clearly indicated.

The laboratory must have a fire prevention policy with written instructions for dealing with an outbreak of fire. Procedures should be reviewed regularly so that changes in structure of premises, installation of new equipment, etc. which may influence fire safety, can be taken into account. The likelihood of fire can be reduced significantly by suitable preventive measures:

1. Good standards of electrical, chemical and general laboratory maintenance.
2. Correct disposal of waste.
3. Correct incineration procedures.
4. Policy of cigarette smoking restriction.
5. Awareness of the possibility of deliberate fire raising.

## **PROCEDURE IN THE EVENT OF FIRE**

### **Protection of life is the first priority**

#### **On discovering a fire:**

Sound the fire alarm and notify the telephone exchange of the exact location of the fire. Attempt to extinguish the fire if you can do so without danger to yourself. If you are unable to do this or you think it too dangerous, then proceed as follows:

- **Make sure that all staff leave the building rapidly but in an orderly manner without using lifts.**

If there is time:

1. Seal any containers of inflammable liquids.
2. Turn off the electricity supply to any equipment or air-conditioning that is in use.
3. Turn off all gas supplies.
4. Close windows.
5. Before leaving the building make sure that each room has been left in a safe condition, and that all rooms, including the toilets and rest room are empty.
6. Make a roll call at the staff assembly point to ensure that all staff are present or accounted for.

Emergency treatment of heat burns: immediately plunge the burned area into cold water or apply a pad soaked in cold water to the affected area for at least 10 minutes. Cover the area with a dry dressing and seek medical aid.

## **ELECTRICAL SAFETY**

Electrical hazards arise from careless or improper use of electrical equipment, incorrect or poorly maintained fittings and connections and from the existence of long trailing leads. Avoidance of electrical hazard can best be achieved by two general policies. The first is only to use electrical equipment which carries a manufacturer's certificate of electrical safety. The second is to insist on installation and maintenance by trained electricians. In addition, cables and plugs should be examined regularly and changed if necessary. Use of extension plugs and boards should be kept to a minimum.

Special precautions are required to ensure safety in the use of electricity where flammable

substances are manufactured, stored, handled, processed or controlled. Electrical installations or apparatus should not be sited in such hazardous areas if it is practical or economic to site them elsewhere. If electrical installations must be sited in hazardous areas, expert advice must be sought on the measures required to prevent fire, explosion and casualties.

Electric shock results from the victim touching a part which is live or charged with electricity so that his body completes the electrical circuit. With high voltages actual contact is not necessary for a current to flow as the current can jump considerable distances. Unless the current which flows to the victim's body is very low, there will be spasm and, in a comparatively short time, death. Ventricular fibrillation is considered to be the principal cause of death; however there is some evidence that asphyxia or cardiac arrest can also occur.

#### **FIRST AID FOR ELECTRIC SHOCK**

- Switch off current - pull out plug
or - Remove victim from danger.

#### **ON NO ACCOUNT ATTEMPT TO REMOVE AN ELECTROCUTED PERSON FROM THE ELECTRICAL CONTACT WITHOUT USING SOME FORM OF INSULATION MATERIAL**

Readily available insulation materials are: folded laboratory coat, folded newspaper, or strip of rubber. Do not use bare hands. A method of using the insulating material is illustrated in Fig. 11.1.

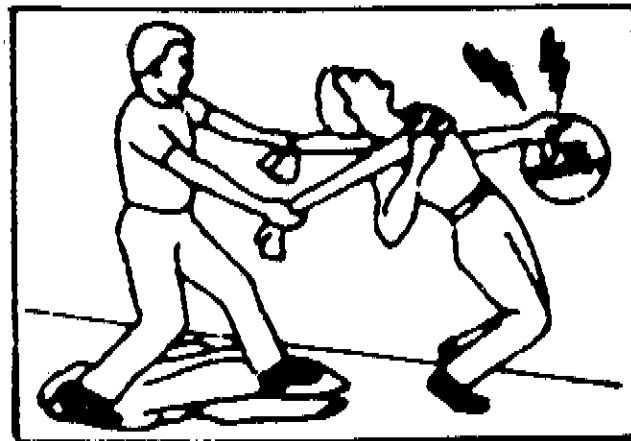


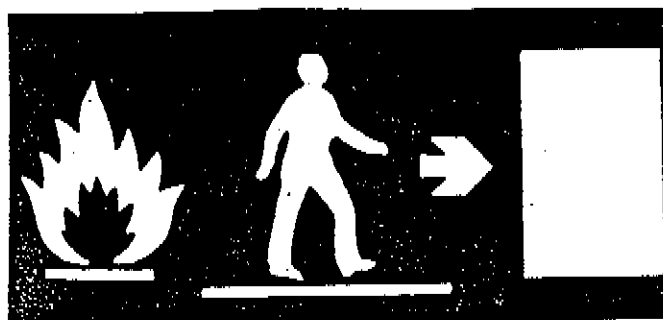
Fig. 11.1

If insulation is not used the person rescuing will also be electrocuted.

If the victim stops breathing following an electrical shock (or for any other reason), artificial respiration must be applied as soon as possible.

### **MICROBIOLOGICAL SAFETY**

All biological materials must be considered potentially infective and must be handled according to established infection-control procedures. It is important to appreciate that persons with pre-existing disease, compromised immunity or who are pregnant may be at additional risk when compared with healthy individuals. For categories of pathogen and required levels of containment the reader is referred to national and international guidelines and Codes of Practice. The CDC/NIH (1984) Biosafety in Microbiological and Medical Laboratories (US Government Printing Office, Washington, D.C., USA) is a useful text. The international biohazard (danger of infection) sign is shown in Fig. 11.2.



**Fig. 11.2 - Biohazard-Danger of infection  
(yellow triangle enclosed by black line with  
yellow notice rectangle)**

Many general microbiological safety measures have already been discussed and can be summarized as follows:

1. All work surfaces should be impervious to water, resistant to chemicals and easily cleaned.
2. Any mechanical ventilation should ensure inward air-flow by extraction.
3. Adequate hand-washing facilities (elbow or foot operating taps) must be provided.
4. Doors must be closed when work is in operation.

5. Approved protective clothing (side or back fastening) must be worn in the laboratory and removed on leaving.
6. Eating, drinking, smoking, storing food and applying cosmetics must be prohibited in the laboratory.
7. Mouth pipetting must be prohibited.
8. Hands must be washed and disinfected immediately whenever contamination by chemical or potentially infectious material occurs and also before leaving the laboratory.
9. All persons handling blood specimens should wear gloves.
10. Masks and protective goggles should be worn when there is a risk of mucous membrane contact with blood.
11. Effective disinfectants must be available.
12. Work surfaces must be cleaned after use.
13. Glassware for disinfection must be stored safely.
14. All potentially infectious waste which is not incinerated must be rendered safe before disposal.
15. Materials for disposal must be transported in robust containers.
16. All laboratory accidents must be reported to and recorded by a responsible person.

## **DISINFECTANTS**

The most commonly encountered dangerous pathogens in haematology and blood transfusion laboratories are hepatitis viruses and HIV. Since phenolic disinfectants are ineffective against these viruses they have been omitted from consideration. As a general rule, hypochlorites are cheap and are the first choice for all but metal surfaces, whereas glutaraldehyde is more expensive but is useful for metal (or metal containing) surfaces or instruments. Both hypochlorite and glutaraldehyde are inactivated by organic material such as blood and therefore have a very limited "bench-life". Disposable gloves should be worn when using any disinfectant. The potential toxicity of glutaraldehyde must be remembered (see below).

### **Hypochlorites (Chlorox, Domestos, DBX)**

These substances attack metal to a varying degree and must not be used on metals or moving parts of machinery. They are suitable for blood and viruses but not for tuberculous material. Commercial products usually contain 100 000ppm available chlorine. They should be used as follows:

1. General use: 1% dilution;
2. Contaminated glassware jars: 2.5% dilution;
3. Blood spillage: 10% dilution.

Fresh working hypochlorite solution must be made daily.



## Aldehydes

These consist of formalin and glutaraldehyde (Cidex). Formalin is a 30% w/v solution of formaldehyde and for general use is diluted 1 in 10 v/v. Formaldehyde gas, prepared by boiling together equal volumes of formalin and water, is useful for disinfecting exhaust protective cabinets but is too irritant for general use. Formaldehyde is used as a fixative in some flow cytometry applications. Formaldehyde mixed with oxidant results in the generation of a carcinogenic substance. For this reason formalin and bleach must not be mixed.

Glutaraldehyde does not readily penetrate organic matter and is most effective on surfaces which have already been cleaned. It is less irritant than formalin and is most useful for disinfecting centrifuges and other metal equipment. It is most efficient at pH 7-8 but deteriorates when alkaline. For use 2% glutaraldehyde is diluted in 0.3% bicarbonate buffer. In recent years, however, the safety of glutaraldehyde has been questioned since there is increasing evidence of its irritant properties particularly to the skin and eyes. In addition, there is evidence that it is hepatotoxic, carcinogenic and teratogenic. An airborne exposure limit of 0.2 ppm has been enforced but when skin contact occurs overexposure may occur within the statutory airborne limit. Sodium perborate containing compounds are now being used as alternatives.

It is often helpful to add a compatible detergent to the disinfectant to be used as this aids cleaning and therefore efficient disinfection, since disinfectants are most active on clean surfaces. There are, however, constraints on this procedure. Hypochlorites are compatible with anionic detergents, e.g., Teepol, HB6 or Triton GR5, and with non-ionic detergents, e.g. Nonidet P40 or Triton X-100, but not with cationic detergents such as cetrimide.

## DECONTAMINATION PROCEDURES

Training must be provided in decontamination procedures. In the event of major spillage of dangerous substances the safety officer must be informed immediately. Four main situations require to be defined.

### Breakage/spillage of specimens

The area of spillage including the broken specimen container should be flooded with appropriate disinfectant. Following disinfectant application the area is left undisturbed for 10 minutes prior to mopping the fluid with an excess of cotton wool or absorbent paper. Disposable gloves, apron and goggles should be worn during the procedure. If a dustpan, brush or forceps are used, these will require disinfection.

### **Breakages within centrifuge**

If breakage is suspected while the centrifuge is still running, the motor must be switched off and the equipment remain closed for 30 minutes. If breakage is discovered on opening the centrifuge, the lid should be replaced immediately and left for 30 minutes. Disposable gloves must be worn. Forceps or cotton wool held in forceps should be used to pick up glass debris. All broken tubes, glass fragments, buckets, trunnions and the rotor must be placed overnight in disinfectant. The centrifuge bowl must then be swabbed with disinfectant, left for 30 minutes, reswabbed, washed with water and dried. Swabs must be treated as infected waste and dealt with accordingly.

### **Chemical spillage**

See section on chemical safety.

### **Decontamination of cell counters**

Manufacturers have the ultimate responsibility for providing appropriate advice regarding decontamination and disinfection of their equipment routinely and prior to visits by service personnel. This responsibility extends to recommendation of appropriate reagents for this purpose and their use. Care should be taken not to mix certain instrument diluents with sodium hypochlorite since they react together and produce chlorine gas. This reaction inactivates the disinfectant making it useless for decontamination. Guidance on this is provided in the manufacturer's manual.

## **CHEMICAL SAFETY**

A number of classes of chemical substance cause hazard in the laboratory.

These classes are grouped as:

- Carcinogens
- Explosive substances
- Dusts
- Solvents
- Acids/corrosives
- Cyanide solutions

The following general management principles apply. A wall chart illustrating the steps to be taken following chemical spillage should be prominently displayed. Gloves, aprons, goggles and appropriate footwear should be worn when dealing with chemical spills.

### Chemical spills

If the amount spilled is small, dilution with water or a detergent will suffice. For large spills, however, spill control bags or absorbent granules should be available. Blood, solvent and acid spills will be considered. A disposable lidded bucket is a suitable container for all three types of spill.

### Blood spill kit

- Disposable gloves, 2 pairs
- Disposable overshoes
- Paper towels, 20 sheets
- Concentrated hypochlorite, 1 bottle (50 ml)
- Water to dilute hypochlorite, 450 ml
- Biohazard card, bearing biohazard label, inscribed "danger, blood spill, keep away".

The lid of the container is labelled as follows:

- Don protective clothing
- Cover area of spill with absorbent paper
- Prepare working solution of hypochlorite
- Pour diluted hypochlorite solution on to absorbant paper covering spill area
- Identify the spill area with the biohazard label (see above)
- Inform a senior person.

### Solvent spill kit

General requirements are similar to blood spill but absorbent granules should be available. Additional instructions include:

- Turn off any naked flames
- Cover area with absorbent granules and allow the spilled fluid to be absorbed
- Use a dustpan and cardboard to pick up the absorbent granules and place in the kit container.

### Acid spill kit

A pot of calcium carbonate powder (1 kg) should be available in the spill kit. The area of the spill should be covered with an excess of powder and allowed to react. When the spill area is neutralized it should be cleaned cautiously using much water: never apply water until the acid has been neutralized.

## REFERENCES

### WHO Documents and Publications

- Fundamental Diagnostic Haematology: Anemia, 1993.
- Fundamental Diagnostic Haematology: The Bleeding and Clotting Disorders, 1993.
- Maintenance and Repair of Laboratory and Hospital Equipment, 1993.
- Self-instruction sheets for users of primary health care laboratory equipment. LAB/85.7, 1985.
- Supply, maintenance and repair of health care laboratory equipment in developing countries. LAB/83.8, 1983.
- Health Laboratory Safety (in preparation).

### WHO/ICSH Documents

- Recommended methods for the determination of packed cell volume by centrifugation. WHO/LAB/89.1, 1989.
- Standardized Romanowsky staining of blood and bone marrow films. WHO/LAB/86.1, 1986.
- Recommended method for the determination of the haemoglobin concentration of blood, WHO/LAB/84.10 Rev. 1, 1991.

### ICSH Publications

- Recommendations for reference method for haemoglobinometry in human blood (ICSH Standard) and specifications for international haemoglobinocyanide reference preparation. *Clinical and Laboratory Haematology* 9: 73-79, 1987.
- The assignment of values to fresh blood used for calibrating automated blood cell counters. *Clinical and Laboratory Haematology* 10: 203-212, 1988.
- ICSH reference method for staining of blood and bone marrow films by azure B and eosin Y (Romanowsky stain). *British Journal of Haematology* 57: 707-710, 1984.
- Recommendation for reference method for determination by centrifugation of packed cell volume of blood. *Journal of Clinical Pathology* 33: 1-2, 1980.

- Recommendation of a system for identifying abnormal haemoglobins. *Blood* 52: 1065-1067, 1978.
- Recommendations for abnormal haemoglobin standard. *British Journal of Haematology* 35: 161-164, 1977.

**Other Publications**

- JV Dacie and SM Lewis. *Practical Haematology* 7th Edn, Churchill Livingstone, Edinburgh, 1991.
- M Cheesborough. *Medical laboratory manual for tropical countries, Volume 1, 2nd Edn, Tropical Health Technology*, 14 Bevills Close, Doddington PE15 OTT, UK, 1987.

= = =