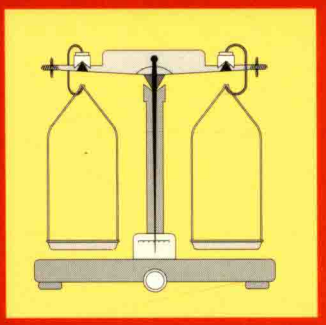
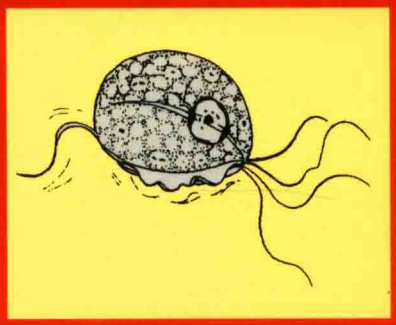
MANUAL OF BASIC TECHNIQUES FOR A HEALTH LABORATORY





2nd edition

World Health Organization
Geneva

Manual of basic techniques for a health laboratory

Second edition

World Health Organization
Geneva
2003

WHO Library Cataloguing-in-Publication Data

Manual of basic techniques for a health laboratory. — 2nd ed.

1.Clinical laboratory techniques — handbooks 2.Technology, Medical — handbooks 3.Manuals

ISBN 92 4 154530 5 (NLM classification: QY 25)

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Design by minimum graphics
Typeset in Hong Kong
Printed in Malta
99/12670 — SNPBest-set/Interprint — 15000

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Preface

This book is a revised edition of the *Manual of basic techniques for a health laboratory* (WHO, 1980), major revisions having been carried out by Dr K. Engbaek, Dr C.C. Heuck and Mr A.H. Moody. The revision was necessary because of new procedures and technology that have been developed since the previous edition and that have proved to be useful to small laboratories in developing countries. The procedures have been included in the relevant sections of the manual, and some obsolete procedures have been replaced by more up-to-date techniques.

The original objective of the manual remains unchanged. It is intended mainly for the use of laboratory personnel in developing countries during their training and thereafter in their work. In the selection of techniques, particular attention has been paid to the low cost, reliability and simplicity of the methods and to the availability of resources in small laboratories.

WHO expresses its thanks to all those who have assisted in the revision of this manual.

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1. Introduction

1.1 Aim of the manual

This manual is intended for use mainly in medical laboratories in developing countries. It is designed particularly for use in peripheral laboratories in such countries (i.e. in small or medium-sized laboratories attached to regional hospitals) and in dispensaries and rural health centres where the laboratory technician often has to work alone. The language used has been kept as simple as possible although common technical terms are employed when necessary.

The manual describes examination procedures that can be carried out with a microscope or other simple apparatus. Such procedures include the following:

— the examination of stools for helminth eggs;

— the examination of blood for malaria parasites;

— the examination of sputum for tubercle bacilli;

— the examination of urine for bile pigments;

— the examination of blood for determination of the white cell (leukocyte) type number fraction (differential leukocyte count)

— the examination of blood for determination of the glucose concentration.

The intention is to provide an account of basic laboratory techniques that are useful to peripheral laboratories and can be carried out with a limited range of basic equipment.

Some laboratories may not be able to perform all the procedures described. For example, a laboratory in a rural health centre may not be able to carry out certain blood chemistry or serological tests.

1.2 Reagents and equipment

1.2.1 Reagents

Each reagent has been given a number. The reagents required and their numbers are indicated in the description of each technique. An alphabetical list of all the reagents used, with the numbers assigned to them, their composition, methods of preparation and storage requirements appears in the Annex at the end of the manual. For example, one of the reagents needed for Gram staining is crystal violet, modified Hucker (reagent no. 18). The composition of crystal violet and the method of preparing it are given in the alphabetical list of reagents (see Annex).

1.2.2 Equipment

The items required for each technique are listed at the beginning of the corresponding section. A list of the apparatus needed to equip a laboratory capable of carrying out all the examinations described in this manual can be found in section 2.5.

When certain articles are not available, the technician should find an appropriate substitute; for example, empty bottles that formerly contained antibiotics for injection ("penicillin bottles") and other drug containers can be kept; racks for test-

tubes and slides can be made locally; and empty tins can be used to make water-baths.

1.3 The responsibility of laboratory workers

Laboratory workers carry out laboratory examinations to provide information for clinical staff in order to benefit patients. They therefore play an important role in helping patients to get better. At the same time, in the course of their work, they gain a lot of information about patients and their illnesses. Laboratory workers, like clinical staff, must regard this information as strictly confidential; only the clinical staff who request the examinations should receive the reports on them. When patients enquire about test results they should be told to ask the clinical staff.

In most countries there are high moral and professional standards of behaviour for clinical staff and qualified laboratory personnel. Every laboratory worker handling clinical materials must maintain these standards.

1.4 Units of measurement

In the laboratory you will work extensively with both quantities and units of measurement, and it is important to understand the difference between them.

Any measurable physical property is called a *quantity*. Note that the word "quantity" has two meanings; the scientific meaning just defined and the everyday meaning "amount of". In scientific usage height, length, speed, temperature and electric current are quantities, whereas the standards in which they are measured are *units*.

1.4.1 Quantities and units in the clinical laboratory

Almost all your work in the laboratory will involve making measurements of quantities and using units for reporting the results of those measurements. Since the health — and even the life — of a patient may depend on the care with which you make a measurement and the way in which you report the results, you should thoroughly understand:

— the quantities you measure;

— the names that are given to those quantities;

— the units that are used to measure the quantities.

1.4.2 SI units and names for quantities

A simple standardized set of units of measurement has been the goal of scientists for almost two centuries. The metric system was introduced in 1901. Since then this system has been gradually expanded, and in 1960 it was given the name "Système international d'Unités" (International System of Units) and the international abbreviation "SI". Units of measurement that form part of this system are called "SI units". These units have been used to an increasing extent in the sciences, especially chemistry and physics, since 1901 (long before they were called SI units), but most of them were introduced into medicine only after 1960.

To accompany the introduction of SI units, medical scientists prepared a systematic list of names for quantities. Some of these names are the same as the traditional ones; in other cases, however, the traditional names were inaccurate, misleading or ambiguous, and new names were introduced to replace them.

This manual uses SI units and the currently accepted names for quantities. However, since traditional units and names for quantities are still used in some laboratories, these are also included and the relationship between the two is explained.

The following section gives a brief description of the SI units and of the quantity names that are used in this manual.

SI units used in this manual

All SI units are based on seven *SI base units*. Only four of them are used in this manual; they are listed in Table 1.1.

Table 1.1 SI base units used in this manual

Quantity	Unit name	Symbol
Length	metre	m
Mass	kilogram	kg
Time	second	s
Amount of substance	mole	mol

The first three of these units will be familiar to you, although the quantity names "mass" and "amount of substance" and the unit name "mole" may need explanation.

Mass is the correct term for what is commonly called "weight". (There is a technical meaning of the term "weight": it is a measure of the force with which the earth's gravity attracts a given mass. Mass, on the other hand, is independent of the earth's gravitational attraction. The two terms are mixed up in everyday usage; furthermore, we speak of measuring a mass as "weighing".) "Amount of substance" and its unit, *mole*, are important terms in medicine and they will affect your work in the laboratory more than any other quantities or SI units. When two or more chemical substances react together, they do not do so in relation to their mass. For example:

$$\begin{matrix}\text{sodium}\\\text{bicarbonate}\end{matrix} + \begin{matrix}\text{hydrochloric}\\\text{acid}\end{matrix} \rightarrow \begin{matrix}\text{sodium}\\\text{chloride}\end{matrix} + \begin{matrix}\text{carbon}\\\text{dioxide}\end{matrix} + \text{water}$$

In this reaction 1 kg (1 kilogram) of sodium bicarbonate does not react with 1 kg of hydrochloric acid; in fact, 1 mol (1 mole) of sodium bicarbonate reacts with 1 mol of hydrochloric acid. Whenever chemical substances interact, they do so in relation to their relative molecular mass (the new name for what used to be called "molecular weight"). Use of the mole, which is based on the relative molecular mass, therefore gives a measure of equivalent amounts of two or more different substances (use of mass units does not).

Most of the SI units are called *SI derived units*. These are obtained by combining the SI base units (by multiplication or division) as appropriate. Some common SI derived units are shown in Table 1.2.

Table 1.2 SI derived units used in this manual

Quantity	Unit name	Symbol
Area	square metre	m^2
Volume	cubic metre	m^3
Speed	metre per second	m/s or ms^{-1}

The unit of area is metre × metre = metre squared or square metre; the unit of volume is metre × metre × metre = metre cubed or cubic metre; and the unit of speed is metre divided by second = metre per second. All the SI derived units are obtained in this simple way. In some cases, however, it is necessary to multiply and

divide several times, and the resulting expression becomes very cumbersome; for example, the unit of pressure is kilogram divided by (metre × second × second). To avoid this difficulty such units are given special names. For example, the unit of pressure is called the pascal.

If the SI base units and derived units were the only ones available, measurements would be difficult because these units are too large or too small for many purposes. For example, the metre is far too large to be convenient for measurement of the diameter of a red blood cell (erythrocyte). To overcome this difficulty, the SI incorporates a series of prefixes, called *SI prefixes*, which when added to the name of a unit multiply or divide that unit by a certain factor, giving decimal multiples or submultiples of the unit. The SI prefixes used in this manual are listed in Table 1.3.

Table 1.3 SI prefixes

Factor	Prefix	Symbol
Multiply by 1 000 000 or 1 million ($\times 10^6$)	mega	M
Multiply by 1000 ($\times 10^3$)	kilo	k
Divide by 100 ($\times 0.01$ or 10^{-2})	centi	c
Divide by 1000 ($\times 0.001$ or 10^{-3})	milli	m
Divide by 1 000 000 ($\times 0.000\,001$ or 10^{-6})	micro	μ
Divide by 1000 million ($\times 0.000\,000\,001$ or 10^{-9})	nano	n

For example, 1 kilometre (1 km) = 1000 metres (1000 m); 1 centimetre (1 cm) = 0.01 metre (0.01 m or 10^{-2} m); 1 millimetre (1 mm) = 0.001 metre (0.001 m or 10^{-3} m); and 1 micrometre (1 μm) = 0.000 001 metre (0.000 001 m or 10^{-6} m). These prefixes have the same meaning when they are applied to any other unit.

Quantity names used in this manual

Certain names for quantities were introduced to accompany the change to SI units. Most of these names are used to describe concentration and related quantities.

Units for measurement of concentration

The difficulty with concentration is that it can be expressed in different ways. Traditionally all of these were called simply "concentration", which was misleading. Now each different way of expressing concentration has its own special name. Before these names can be described, it is necessary to explain the unit of volume called the "litre" (l). You are probably familiar with this unit of volume, and may have been surprised that it has not already been mentioned. This is because the litre is not an SI unit.

The SI derived unit of volume is the cubic metre, but this is far too large to be convenient for measurements of body fluids. A submultiple of the cubic metre is therefore used; the cubic decimetre. The prefix "deci" was not listed above because it is not used in this manual, but it means division by 10 (or multiplication by 0.1 or 10^{-1}). A decimetre is therefore 0.1 m, and a cubic decimetre is $0.1 \times 0.1 \times 0.1\,m^3 = 0.001\,m^3$ (or $10^{-3}\,m^3$; that is, one-thousandth of a cubic metre). The name "litre", although not part of the SI, has been approved for use as a special name for the cubic decimetre. The litre and its submultiples, such as the millilitre (ml), are used mainly for measuring relatively small volumes of liquids and sometimes gases; volumes of solids and large volumes of liquids and gases are usually measured in terms of the cubic metre or one of its multiples or submultiples. The litre is the unit used in the clinical laboratory for reporting all concentrations and related quantities. However, you may encounter (for example, on graduated glassware) volumes

Table 1.4 SI derived units of volume

Unit name	Symbol	Equivalent in cubic metres (m^3)	Unit name	Symbol	Equivalent in litres (l)	Equivalent in millilitres (ml)
Cubic decimetre	dm^3	0.001	litre	l	1	1000
—	$100\,cm^3$	0.0001	decilitre[a]	dl	0.1	100
—	$10\,cm^3$	0.000 01	centilitre[a]	cl	0.01	10
Cubic centimetre	cm^3	0.000 001	millilitre	ml	0.001	1
Cubic millimetre	mm^3	0.000 000 001	microlitre	µl	0.000 001	0.001

[a] Seldom used in the laboratory.

marked in terms of submultiples of the cubic metre. The equivalent submultiples of the cubic metre and of the litre are listed in Table 1.4.

Having explained the litre, we can now return to the names for different ways of expressing concentration. First, suppose that we have a solution of salt. The mass of dissolved salt divided by the volume of solution is called the *mass concentration*. A more general definition of mass concentration is "the mass of a given component (e.g. a dissolved substance) divided by the volume of solution". The unit in which it is measured is gram (or milligram, microgram, etc.) per litre. In the SI mass concentration is rarely used; it is used only for substances such as proteins whose relative molecular mass is uncertain.

Now suppose that we have another solution of salt, only this time the amount of dissolved salt is expressed in terms of the "amount of substance". The amount of substance of salt (that is, the number of moles of salt) contained in the solution divided by the volume of the solution is called the amount of substance concentration, or, for short, the *substance concentration*. The unit in which substance concentration is measured is mole (or millimole, micromole, etc.) per litre. When SI units are used all concentrations are expressed in terms of substance concentration wherever possible.

This use of substance concentration instead of mass concentration is the most important difference between the use of SI units and the use of traditional units.

> In the traditional system mass concentration was used almost exclusively. However, mass concentration was not, in the traditional system, always expressed in terms of "per litre". Sometimes "per litre" was used, sometimes "per 100 ml" (0.1 litre), and sometimes "per millilitre". Different countries (and even different laboratories in the same country) followed different practices, making for considerable confusion.

For particles or entities that are not dissolved, a different quantity must be used. For example, the blood contains many different kinds of cell. These cells are suspended in the blood, and we must have a way of expressing the number of cells in each litre of blood. In this case the quantity name is the *number concentration*, which is defined as "the number of specified particles or entities in a mixture divided by the volume of the mixture". The unit in which number concentration is measured is number per litre.

> In the traditional system number concentration was called a "count" and it was expressed in the unit "number per cubic millimetre".

Sometimes the quantity that is of concern is not the actual number of cells per litre (number concentration) but the proportion of cells of a given type — that is, the fraction of the total number that is accounted for by cells of that type. This quantity is called the *number fraction*, and it is expressed as a fraction of 1.0 (unity). At first sight this may seem a little confusing, but it is really very simple. Unity or 1.0 represents the whole, 0.5 represents one-half, 0.2 one-fifth, 0.25 one-quarter, 0.1 one-tenth, and so on. For example, five kinds of leukocyte occur in the blood. The

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