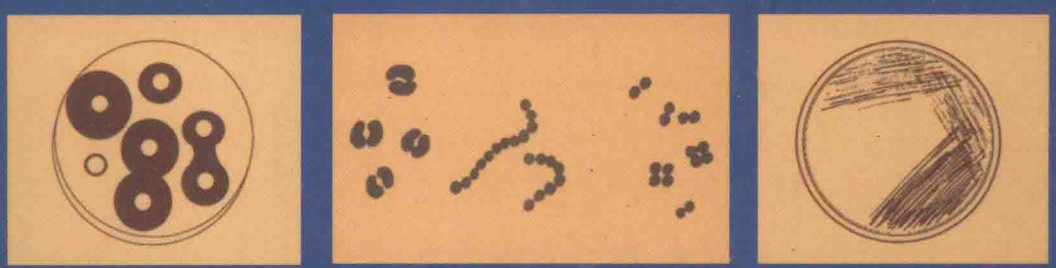
BASIC LABORATORY PROCEDURES



IN CLINICAL BACTERIOLOGY

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Basic laboratory procedures in clinical bacteriology

Second edition

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Basic laboratory procedures in clinical bacteriology

Preface

Communicable diseases are the most common cause of death in developing countries, and their diagnosis and treatment represent a significant challenge to the health services in those areas. The World Health Organization has long been actively involved in developing and promoting standard techniques for laboratory investigations of such diseases, a first attempt to standardize susceptibility testing of bacterial pathogens being made in 1960.[1] Following on from this, in 1976, the WHO Expert Committee on Biological Standardization drew up requirements for antibiotic susceptibility testing using the disc method.[2]

At the same time, efforts were being made to introduce quality control into laboratory performance. In 1981, WHO established an International External Quality Assessment Scheme for Microbiology. The laboratories that are involved in this scheme are able to play a leading role in the implementation of national quality assessment schemes at all levels of the health care system.

The present publication brings together and updates the various guidelines produced by WHO over the years on sampling of specimens for laboratory investigation, identification of bacteria, and testing of antimicrobial resistance. The information included is intended to lead to harmonization of microbiological investigations and susceptibility testing, and to improve the quality of laboratories at both central and intermediate levels. It concentrates on the procedures to be followed, rather than the basic techniques of microscopy and staining, which have been described in detail in another WHO publication.[3]

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[1] *The public health aspects of antibiotics in feedstuffs. Report on a Working Group, Bremen, 1–5 October 1973*. Copenhagen, WHO Regional Office for Europe, 1973 (document no. EURO 3604 (2)).

[2] *WHO Expert Committee on Biological Standardization. Twenty-eighth report*. Geneva, World Health Organization, 1977 (WHO Technical Report Series, No. 610).

[3] *Manual of basic techniques for a health laboratory*, 2nd ed. Geneva, World Health Organization, 2003.

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Introduction

Communicable diseases continue to account for an unduly high proportion of the health budgets of developing countries. According to *The world health report*,[1] acute diarrhoea is responsible for as many as 2.2 million deaths annually. Acute respiratory infections (primarily pneumonia) are another important cause of death, resulting in an estimated 4 million deaths each year. Analysis of data on lung aspirates appears to indicate that, in developing countries, bacteria such as *Haemophilus influenzae* and *Streptococcus pneumoniae*, rather than viruses, are the predominant pathogens in childhood pneumonia. β-Lactamase-producing *H. influenzae* and *S. pneumoniae* with decreased sensitivity to benzylpenicillin have appeared in different parts of the world, making the surveillance of these pathogens increasingly important.

Sexually transmitted diseases are on the increase. There are still threats of epidemics and pandemics of viral or bacterial origin, made more likely by inadequate epidemiological surveillance and deficient preventive measures. To prevent and control the main bacterial diseases, there is a need to develop simple tools for use in epidemiological surveillance and disease monitoring, as well as simplified and reliable diagnostic techniques.

To meet the challenge that this situation represents, the health laboratory services must be based on a network of laboratories carrying out microbiological diagnostic work for health centres, hospital doctors, and epidemiologists. The complexity of the work will increase from the peripheral to the intermediate and central laboratories. Only in this way will it be possible to gather, quickly enough, sufficient relevant information to improve surveillance, and permit the early recognition of epidemics or unusual infections and the development, application, and evaluation of specific intervention measures.

[1] *The world health report 2000*. Geneva, World Health Organization, 2000.

Quality assurance in bacteriology

Introduction

Quality assurance programmes are an efficient way of maintaining the standards of performance of diagnostic laboratories, and of upgrading those standards where necessary. In microbiology, quality goes beyond technical perfection to take into account the speed, cost, and usefulness or clinical relevance of the test. Laboratory tests in general are expensive and, with progress in medicine, they tend to use up an increasing proportion of the health budget.

Definitions

To be of good quality, a diagnostic test must be clinically relevant, i.e. it must help in the prevention or treatment of disease. Other measures of quality in a diagnostic test are:

- *Reliability*: Is the result correct?
- *Reproducibility*: Is the same result obtained when the test is repeated?
- *Speed*: Is the test rapid enough to be of use to the doctor in prescribing treatment?
- *Cost–benefit ratio*: Is the cost of the test reasonable in relation to the benefit to the patient and the community?

Factors that affect the reliability and reproducibility of laboratory results

Sources of error may include the following:

- *Personnel*. The performance of the laboratory worker or technician is directly related to the quality of education and training received, the person's experience, and the conditions of employment.
- *Environmental factors*. Inadequate working space, lighting, or ventilation, extreme temperatures, excessive noise levels, or unsafe working conditions may affect results.
- *Specimens*. The method and time of sampling and the source of the specimen are often outside the direct control of the laboratory, but have a direct bearing on the ability of the laboratory to achieve reliable results. Other factors that the laboratory can control and that affect quality are the transport, identification, storage, and preparation (processing) of specimens. The laboratory therefore has a role in educating those taking and transporting specimens. Written instructions should be made available and regularly reviewed with the clinical and nursing staff.
- *Laboratory materials*. The quality of reagents, chemicals, glassware, stains, culture media, and laboratory animals all influence the reliability of test results.
- *Test method*. Some methods are more reliable than others.
- *Equipment*. Lack of equipment or the use of substandard or poorly maintained instruments will give unreliable results.
- *Examination and reading*. Hurried reading of results, or failure to examine a sufficient number of microscope fields, can cause errors.
- *Reporting*. Transcription errors, or incomplete reports, cause problems.

Quality of interpretation of test results

Interpretation is of particular importance in microbiology. At each stage in the examination of a specimen, the results should be interpreted in order to select the optimum test, in terms of speed and reliability, for the next stage of the examination.

Quality assurance in the microbiology laboratory

Quality assurance is the sum of all those activities in which the laboratory is engaged to ensure that test results are of good quality. It must be:

— *comprehensive*: to cover every step in the cycle from collecting the specimen to sending the final report to the doctor (Fig. 1);
— *rational*: to concentrate on the most critical steps in the cycle;
— *regular*: to provide continuous monitoring of test procedures;
— *frequent*: to detect and correct errors as they occur.

GOOD-QUALITY LABORATORY SERVICES MEAN GOOD-QUALITY MEDICINE

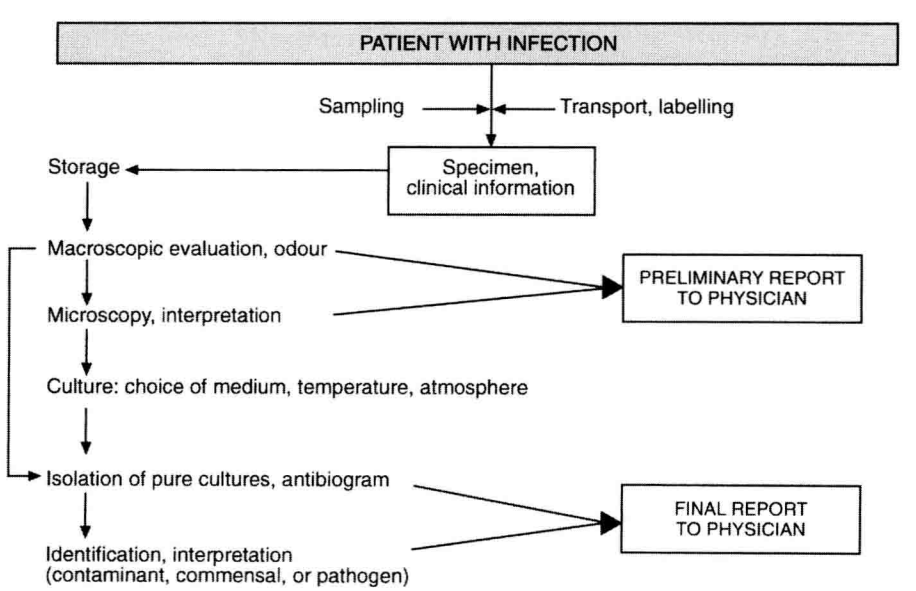
Quality assurance helps to ensure that expensive tests are used as economically as possible; it also determines whether new tests are valid or worthless, improves the performance of clinical and public health laboratories, and may help to make the results obtained in different laboratories comparable.

Types of quality assurance

There are two types of quality assurance: internal and external.

- *Internal*. This is called QUALITY CONTROL. Each laboratory has a programme to check the quality of its own tests.

Fig. 1. Steps in laboratory investigation of an infected patient



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Internal quality control involves, ideally:

— *continuous monitoring* of test quality;
— *comprehensive checking* of all steps, from collecting the specimen (whenever possible) to sending the final report.

Laboratories have an ethical responsibility to the patient to produce accurate, meaningful results.

INTERNAL QUALITY CONTROL IS ABSOLUTELY ESSENTIAL FOR GOOD OPERATING PROCEDURE

- *External.* This is called QUALITY ASSESSMENT. Laboratory performance is controlled by an external agency. In some countries, participation is mandatory (regulated by the government) and required for licensure.

External quality assessment involves:

— *periodic monitoring* of test quality;
— *spot checking* of identification tests, and sometimes of isolation techniques.

Quality criteria in microbiology

Clinical relevance

An important criterion of quality for a microbiological test is how much it contributes to the prevention or cure of infectious diseases; this is called its clinical relevance. Clinical relevance can only be ensured when there is good communication between the clinician and the laboratory.

To illustrate clinical relevance, here are some examples:

1. If a few colonies of Gram-negative rods are isolated from the sputum or throat swab of a hospitalized patient, further identification and an antibiogram are of no clinical relevance, since neither procedure will have any effect on treatment of the patient.

2. If *Streptococcus pyogenes* is isolated, a full antibiogram has no clinical relevance, since benzylpenicillin is the drug of choice, and this is always active in vitro.

3. If *Escherichia coli* is isolated from a sporadic case of non-bloody diarrhoea, identification of the serotype is of no clinical relevance, since there is no clearly established correlation between serotype and pathogenicity.

4. If a Gram-stained smear shows "mixed anaerobic flora", routine identification of the anaerobes is of no clinical relevance. It would be costly in time and materials, and would not affect treatment of the patient.

5. If a yeast is isolated from a respiratory tract specimen, an identification test for *Cryptococcus* should be done. Further identification tests have no clinical relevance, since they would have no effect on patient management.

In summary, a test of good quality is one that is accurate and gives useful results for the prevention or cure of infection. It is not necessary to isolate and identify all the different types of organism in the sample.

Reliability

For tests that give quantitative results, reliability is measured by how close the results are to the true value. Some examples of tests of this kind are:

— antibiotic assay of serum;
— measurement of minimal inhibitory concentration (MIC) values of antibiotics in vitro;
— serum antibody titrations.

For tests that give qualitative results, reliability is measured by whether the result is correct. Some examples of tests of this kind are:

— identification of pathogens;
— antibiotic susceptibility testing of isolates by the disc method.

Standard terminology for microorganisms is essential to reliability. Internationally recognized nomenclature should always be used. For example: *Staphylococcus aureus*, NOT "pathogenic staphylococci"; *Streptococcus pyogenes*, NOT "haemolytic streptococci".

Use of uniform, approved methods is essential. For example, disc susceptibility tests should be performed with an internationally recognized technique, such as the modified Kirby–Bauer test (page 109).

Reproducibility

The reproducibility or precision of a microbiological test is reduced by two things:

1. *Lack of homogeneity*. A single sample from a patient may contain more than one organism. Repeat culturing may therefore isolate different organisms.
2. *Lack of stability*. As time passes, the microorganisms in a specimen multiply or die at different rates. Repeat culturing may therefore isolate different organisms. To improve precision, therefore, specimens should be tested as soon as possible after collection.

Efficiency

The efficiency of a microbiological test is its ability to give the correct diagnosis of a pathogen or a pathological condition. This is measured by two criteria:

1. *Diagnostic sensitivity*

$$\text{Sensitivity} = \frac{\text{total number of positive results}}{\text{total number of infected patients}}$$

The greater the sensitivity of a test, the fewer the number of false-negative results.

For example, the sensitivity of MacConkey agar is poor for the isolation of *Salmonella typhi* from stool. This important enteric pathogen is often missed because of overgrowth by nonpathogenic intestinal bacteria.

2. *Diagnostic specificity*

$$\text{Specificity} = \frac{\text{total number of negative results}}{\text{total number of unifected patients}}$$

The greater the specificity of a test, the fewer the number of false-positive results.

For example:

- Ziehl–Neelsen staining of sputum is highly specific for diagnosing tuberculosis, because it gives only a few false-positive results.
- Ziehl–Neelsen staining of urine is much less specific, because it gives many false-positive results (as a result of atypical mycobacteria).
- The Widal test has a very low specificity for the diagnosis of typhoid fever, because cross-agglutinating antibodies remaining from past infections with related salmonella serotypes give false-positive results.

The sensitivity and specificity of a test are interrelated. By lowering the level of discrimination, the sensitivity of a test can be increased at the cost of reducing its specificity, and vice versa. The diagnostic sensitivity and specificity of a test are also related to the prevalence of the given infection in the population under investigation.

Internal quality control

Requirements

An internal quality control programme should be practical, realistic, and economical.

An internal quality control programme should not attempt to evaluate every procedure, reagent, and culture medium on every working day. It should evaluate each procedure, reagent, and culture medium according to a practical schedule, based on the importance of each item to the quality of the test as a whole.

Procedures

Internal quality control begins with proper laboratory operation.

Laboratory operations manual

Each laboratory should have an operations manual that includes the following subjects:

— cleaning of the working space,
— personal hygiene,
— safety precautions,
— designated eating and smoking areas located outside the laboratory,
— handling and disposal of infected material,

— appropriate vaccinations for workers, e.g. hepatitis B,
— care of equipment,
— collection of specimens,
— registration of specimens,
— elimination of unsuitable specimens,
— processing of specimens,
— recording of results,
— reporting of results.

The operations manual should be carefully followed, and regularly revised and updated.

Care of equipment

It is particularly important to take good care of laboratory equipment. Good quality tests cannot be performed if the equipment used is either of poor quality or poorly maintained.

Table 1 is a schedule for the routine care and maintenance of essential equipment. Equipment operating temperatures may be recorded on a form such as the one shown in Fig. 2.

Culture media

Culture media may be prepared in the laboratory from the basic ingredients or from commercially available dehydrated powders, or they may be purchased ready for use. Commercial dehydrated powders are recommended because they are economical to transport and store, and their quality is likely to be higher than media prepared in the laboratory. For best results, careful attention is required to the points itemized below.

Selection of media

An efficient laboratory stocks the smallest possible range of media consistent with the types of test performed. For example, a good agar base can be used as an all-purpose medium for preparing blood agar, chocolate agar, and several selective media.

One highly selective medium (*Salmonella–Shigella* agar or deoxycholate citrate agar) and one less selective medium (MacConkey agar) are necessary for the isolation of pathogenic Enterobacteriaceae from stools.

A special culture medium should be added for the recovery of *Campylobacter* spp.

Ordering and storage of dehydrated media

1. Order quantities that will be used up in 6 months, or at most 1 year.
2. The overall quantity should be packed in containers that will be used up in 1–2 months.
3. On receipt, tighten caps of all containers securely. Dehydrated media absorb water from the atmosphere. In a humid climate, seal the tops of containers of dehydrated media with paraffin wax (fill the space between the lid and container with molten wax, and let it harden).

Table 1. Quality control of equipment

Equipment	Routine care	Monitoring	Technical maintenance and inspection
Anaerobic jar	Clean inside of jar each week Reactivate catalyst after each run (160 °C, 2 h) Replace catalyst every 3 months	Use methylene blue indicator strip with each run Note and record decolorization time of indicator each week	Inspect gasket sealing in the lid weekly
Autoclave	Clean and change water monthly	Check and adjust water level before each run Record time and temperature or pressure for each run Record performance with spore-strips weekly	Every 6 months
Centrifuge	Wipe inner walls with antiseptic solution weekly or after breakage of glass tubes or spillage		Replace brushes annually
Hot-air oven for sterilization of glassware	Clean inside monthly	Record time and temperature for each run	Every 6 months
Incubator	Clean inside walls and shelves monthly	Record temperature at the start of each working day (allowance 35 ± 1 °C)	Every 6 months
Microscope	Wipe lenses with tissue or lens paper after each day's work Clean and lubricate mechanical stage weekly Protect with dust cover when not in use	Check alignment of condenser monthly Place a dish of blue silica with the microscope under the dust cover to prevent fungal growth in humid climates	Annually
Refrigerator	Clean and defrost every 2 months and after power failure	Record temperature every morning (allowance 2–8 °C)	Every 6 months
Water-bath	Wipe inside walls and change water monthly	Check water level daily Record temperature on first day of each week (allowance 55–57 °C)	Every 6 months

4. Write the date of receipt on each container.
5. Store in a dark, cool, well-ventilated place.
6. Rotate the stock so that the older materials are used first.
7. When a container is opened, write the date of opening on it.
8. Discard all dehydrated media that are either caked or darkened.
9. Keep written records of media in stock.

Preparation of media

1. Follow strictly the manufacturer's instructions for preparation.
2. Prepare a quantity that will be used up before the shelf-life expires (see below).

Fig. 2. Record of equipment operating temperature

Temperature _____

Instrument _____ Room _____

Read daily. Check if temperature indication is acceptable. If aberrant, record temperature in space.

Date	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Date
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