B A S I C LABORATORY PROCEDURES







IN CLINICAL BACTERIOLOGY

2nd edition



World Health Organization Geneva

Basic laboratory procedures in clinical bacteriology

Second edition

J. Vandepitte and J. Verhaegen Department of Microbiology St Rafaël Academic Hospital Leuven, Belgium

K. Engbaek

Department of Clinical Microbiology University of Copenhagen Herlev Hospital Herlev, Denmark

P. Rohner

Department of Clinical Microbiology Cantonal University Hospital Geneva, Switzerland

P. Piot

Joint United Nations Programme on HIV/AIDS Geneva, Switzerland

C. C. Heuck

World Health Organization Geneva, Switzerland



World Health Organization Geneva 2003 WHO Library Cataloguing-in-Publication Data

Basic laboratory procedures in clinical bacteriology / J. Vandepitte . . . [et al.]. - 2nd ed.

1.Bacteriological techniques—standards 2.Laboratory techniques and procedures standards 3.Manuals I.Vandepitte, J.

ISBN 92 4 154545 3

(NLM classification: QY 100)

© World Health Organization 2003

All rights reserved. Publications of the World Health Organization can be obtained from Marketing and Dissemination, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel: +41 22 791 2476; fax: +41 22 791 4857; email: bookorders@who.int). Requests for permission to reproduce or translate WHO publications—whether for sale or for noncommercial distribution—should be addressed to Publications, at the above address (fax: +41 22 791 4806; email: permissions@who.int).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

The World Health Organization does not warrant that the information contained in this publication is complete and correct and shall not be liable for any damages incurred as a result of its use.

The named authors alone are responsible for the views expressed in this publication.

Typeset in Hong Kong Printed in Singapore 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. Following on from this, in 1976, the WHO Expert Committee on Biological Standardization drew up requirements for antibiotic susceptibility testing using the disc method. Expert Committee on Biological Standardization drew up requirements for antibiotic susceptibility testing using the disc method.

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

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)).

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.

Contents

Introduction Quality assurance in bacteriology Introduction Definitions Internal quality control External quality assessment PART I Bacteriological investigations Blood Introduction When and where bacteraemia may occur Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Microscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Media for enteric pathogens Primary isolation Preliminary identification of specimens Apprimary isolation Etiorication and transport of stool specimens Media for enteric pathogens Primary isolation Preliminary identification of isolates 44	Preface	viii
Introduction Definitions Internal quality control External quality assessment PART I Bacteriological investigations Blood Introduction When and where bacteraemia may occur Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic examination Preliminary identification Susceptibility testing Urine Introduction Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Media for enteric pathogens Primary isolation 42 PART I Bacteriological investigations 15 26 27 28 29 29 20 20 20 21 22 23 24 25 26 27 28 29 29 20 20 20 20 20 20 20 20	Introduction	1
Definitions Internal quality control External quality assessment PART I Bacteriological investigations Blood Introduction When and where bacteraemia may occur Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Visual examination of stool specimens Primary isolation 42 44 45 46 47 48 48 49 40 40 41 42 44 44 44 44 44 44 44 44	Quality assurance in bacteriology	2
Definitions Internal quality control External quality assessment PART I Bacteriological investigations Blood Introduction When and where bacteraemia may occur Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic examination Preliminary identification Susceptibility testing Urine Introduction Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Visual examination of stool specimens Primary isolation Preliminary isolation 42 Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Primary isolation 42 Appropriate use of laboratory of stool specimens Primary isolation 44 Approprimary isolation	Introduction	2
External quality assessment PART I Bacteriological investigations Introduction When and where bacteraemia may occur Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Visual examination of stool specimens Visual examination of stool specimens Primary isolation 19 20 20 21 22 25 26 27 28 29 20 20 21 21 22 23 24 24 25 26 27 28 28 28 28 29 20 20 20 20 20 20 20 20 20 20 20 20 20		2
PART I Bacteriological investigations Blood Introduction When and where bacteraemia may occur Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Visual primary isolation	Internal quality control	
Blood Introduction When and where bacteraemia may occur Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Visual examination of stool specimens Visual examination of stool specimens Primary isolation Page 192 Value 202 Value 202 Value 203 Value	External quality assessment	16
Blood Introduction When and where bacteraemia may occur Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Visual examination of stool specimens Visual examination of stool specimens Primary isolation Page 22 22 25 26 27 28 29 20 20 21 22 23 24 25 26 27 28 29 20 20 20 20 20 20 20 20 20		
Introduction When and where bacteraemia may occur Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Stool Introduction Stool Stool Stool Introduction Stool	Bacteriological investigations	19
When and where bacteraemia may occur Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Media for enteric pathogens Primary isolation 22 23 24 25 26 27 28 29 29 20 20 21 22 21 22 23 24 25 26 27 27 28 28 29 20 20 21 21 22 23 24 25 26 27 27 28 28 29 20 20 21 21 21 22 23 24 25 26 27 27 28 28 29 20 20 20 21 21 21 21 22 23 24 25 26 27 27 28 28 29 20 20 21 21 21 21 21 21 21 21 21 21 21 21 21	Blood	20
When and where bacteraemia may occur Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Media for enteric pathogens Primary isolation 225 237 248 258 269 279 280 281 281 282 283 284 285 286 287 288 288 288 288 288 288 288 288 288	Introduction	20
Blood collection Blood-culture media Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Stool Introduction Susceptibility tests Stool Introduction Stool Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation		20
Processing of blood cultures Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation		20
Cerebrospinal fluid Introduction Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Stool Stool Stool Introduction Stool Specimens Stool Stool Specimens Spe	Blood-culture media	22
Introduction 25 Collection and transportation of specimens 25 Macroscopic inspection 26 Microscopic examination 26 Preliminary identification 28 Susceptibility testing 29 Urine 30 Introduction 30 Specimen collection 30 Interpretation of quantitative urine culture results 34 Identification 35 Identification 35 Susceptibility tests 36 Stool 37 Introduction 37 Etiological agents and clinical features 37 Appropriate use of laboratory resources 39 Collection and transport of stool specimens 40 Visual examination of stool specimens 41 Enrichment and inoculation of stool specimens 42 Primary isolation 42	Processing of blood cultures	23
Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Media for enteric pathogens Primary isolation 26 26 27 28 30 30 30 31 32 33 34 35 36 37 37 37 37 48 49 40 40 40 40 40 40 40 40 40	Cerebrospinal fluid	25
Collection and transportation of specimens Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Stool Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Media for enteric pathogens Primary isolation 25 26 27 37 37 37 37 37 47 47 47 47 4	Introduction	25
Macroscopic inspection Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Stool Stool Stool Introduction Stool S		25
Microscopic examination Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Stool Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 28 29 29 20 21 22 25 26 27 27 28 29 20 20 20 21 21 22 22 22 23 24 25 26 27 27 28 28 29 20 20 20 20 20 21 21 22 22 22 22 22 22 22 22 22 22 22		26
Preliminary identification Susceptibility testing Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Henrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 28 29 29 29 20 20 21 21 22 22 22 24 25 26 27 27 28 28 28 29 20 20 21 21 22 22 22 22 22 22 22 22 22 22 22		26
Urine Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 29 30 30 31 32 34 35 36 37 40 41 42 42 44 44 44 44 44 44 44		28
Introduction Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 30 30 31 32 32 34 35 35 36 37 37 37 47 48 48 48 48 48 48 48 48 48 48 48 48 48		29
Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 32 33 35 36 37 37 38 38 39 39 30 30 30 30 30 30 30 30 30 30 30 30 30	Urine	30
Specimen collection Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 32 33 35 36 37 37 38 38 39 39 30 30 30 30 30 30 30 30 30 30 30 30 30	Introduction	30
Culture and interpretation Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 32 33 35 36 37 37 38 38 39 39 30 30 30 30 30 30 30 30 30 30 30 30 30		
Interpretation of quantitative urine culture results Identification Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 33 44 45 46 47 48 48 48 48 48 48 48 48 48		
Identification Susceptibility tests Stool Introduction Stiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 35 36 37 37 38 38 38 38 38 38 38 38 38 38 38 38 38		
Susceptibility tests Stool Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 37 48 49 40 41 42 42 43		35
Introduction Etiological agents and clinical features Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens 41 Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 37 40 41 42 42		
Etiological agents and clinical features 37 Appropriate use of laboratory resources 39 Collection and transport of stool specimens 40 Visual examination of stool specimens 41 Enrichment and inoculation of stool specimens 41 Media for enteric pathogens 42 Primary isolation 42	Stool	37
Etiological agents and clinical features 37 Appropriate use of laboratory resources 39 Collection and transport of stool specimens 40 Visual examination of stool specimens 41 Enrichment and inoculation of stool specimens 41 Media for enteric pathogens 42 Primary isolation 42	Introduction	37
Appropriate use of laboratory resources Collection and transport of stool specimens Visual examination of stool specimens Enrichment and inoculation of stool specimens Media for enteric pathogens Primary isolation 39 40 41 42		
Collection and transport of stool specimens 40 Visual examination of stool specimens 41 Enrichment and inoculation of stool specimens 41 Media for enteric pathogens 42 Primary isolation 42		
Visual examination of stool specimens 41 Enrichment and inoculation of stool specimens 41 Media for enteric pathogens 42 Primary isolation 42		
Enrichment and inoculation of stool specimens 41 Media for enteric pathogens 42 Primary isolation 42	•	
Media for enteric pathogens 42 Primary isolation 42		
Primary isolation 42		
·	. •	42
		44

Final microbiological identification Serological identification	50 54
Upper respiratory tract infections	60
Introduction	60
Normal flora of the pharynx	60
Bacterial agents of pharyngitis	61
Collection and dispatch of specimens	62
Direct microscopy	62
Culture and identification	63
Susceptibility testing	65
Lower respiratory tract infections	66
Introduction	66
The most common infections	66
Collection of sputum specimens	68
Processing of sputum in the laboratory (for	
non-tuberculous infections)	68
Culture for Mycobacterium tuberculosis	72
Interpretation of cultures for M. tuberculosis	74
General note on safety	74
Sexually transmitted diseases	76
Introduction	76
Urethritis in men	77
Genital specimens from women	79
Specimens from genital ulcers	82
Purulent exudates, wounds and abscesses	86
Introduction	86
Commonly encountered clinical conditions and the	
most frequent etiological agents	86
Collection and transportation of specimens	89
Macroscopic evaluation	90
Microscopic examination	91
Culture	92
Identification	93
Susceptibility testing	97
Anaerobic bacteriology	98
Introduction	98
Description of bacteria in relation to oxygen requirement	98
Bacteriology	98
Antimicrobial susceptibility testing	103
Introduction	103
General principles of antimicrobial susceptibility testing	103
Clinical definition of terms "resistant" and "susceptible":	
the three category system	104
Indications for routine susceptibility tests	106

Choice of drugs for routine susceptibility tests in the clinical laboratory	107
The modified Kirby-Bauer method	109
Direct versus indirect susceptibility tests	117
Technical factors influencing the size of the zone in the	
disc-diffusion method	118
Quality control	120
Serological tests	122
Introduction	122
Quality control measures	122
Serological reactions	125
Serological tests for syphilis	126
Febrile agglutinins tests	133
Antistreptolysin O test	135
Bacterial antigen tests	137
PART II Essential media and reagents	141
Introduction	142
Pathogens, media and diagnostic reagents	143
Blood	144
Cerebrospinal fluid	144
Urine	145
Stool	146
Upper respiratory tract	147
Lower respiratory tract	148
Urogenital specimens for exclusion of sexually transmitted	
diseases	149
Pus and exudates	149
List of recommended media and diagnostic reagents	
for the intermediate microbiological laboratory	150
Selected further reading	154
Index	155

Introduction

Communicable diseases continue to account for an unduly high proportion of the health budgets of developing countries. According to *The world health report*, 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

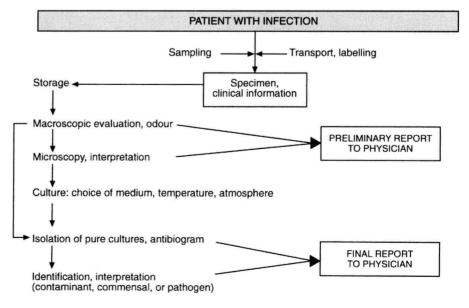
Quality assurance helps to ensure that expensive tests are used as economically as possible; it also determines whether new tests are valid or worthless, improves the performance of clinical and public health laboratories, and may help to make the results obtained in different laboratories comparable.

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



WHO 90960

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:

- 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

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

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, 2h) 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	n is acceptable. If aberrant, record temper-
ature in space.	

ature	in spa	ice.											
Date	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Date
1		l !	I !	1 1		 		 	l :		 		1
2		 -		+ !	 	 	 	 	 		 		2
3		 		 ! •	 	,		, 			 		3
4				! !	 	! !		I I					4
5			 	! +		 	 	 			 		5
6		 	 	 	 	I I – – -	 	l I	 		 	l	6
7		 	 	l +	l 	I I – – -	! +	I I	 +		 -		7
8		 	i 1 – – -	 - -	l 	 	·	 	 		 		8
9		 	 	 	 	 	 	 	 +		 		9
10		 -	l 	 	 	 +	 	 -	 +		 		10
11			 	 	 	 		 -	 +		 		11
12			 	 	! 	 +		 	! ! !+		 		12
13			(l 			 	 +	1	 -		13
14			 						+		 		14
15			 	 	- 	4			 +		 -		15
16			1				= =		+		I		16
17									 		i		17
18 19			 	1		4		i	+	i	 		18
20	i			i		 		i	+	j	i		19 20
21	i		#	i	i	 	i	i	-	i	i		21
22	1		 	i	i	1	i	i	i	i	i		22
23	I		+ 	I	I I	+ 1	I	I	i	i	<u> </u>		23
24	I		+ 		I	+	I	I	+	I	ı		24
25	I		+ 	I	I I	+ I	I	I	+ 1	I	I		25
26	I		+ 	1	! !	+ I	I	I I	+	J	l I		26
27	I	-	+ I	1	I	+ I	I		+ 1	1 1	I		27
28	J-	I	+	I	I I	+ (I	1	+ I	I	 I		28
29	I	I	+ I	I		+ !	I	(+ I	1	I I		29
30		I I	+	E	1	+	I	I I	+ 1	1-	I I		30
31	I-	I I	+	1	I	+ 1	I	I	+ !	I-			31
		1		1		1		Î	1				