WHO/CDS/EPR/LYO/2007.3

Policy and procedures of the

WHO/NICD Microbiology External Quality Assessment Programme in Africa

Years 1 to 4
2002-2006

EPIDEMIC AND PANDEMIC
ALERT AND RESPONSE

World Health Organization

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Acknowledgments

In addition to the invaluable roles played by the participating laboratories and referee laboratories, WHO would like to acknowledge with gratitude the contributions of the following to the development and implementation of the microbiology External Quality Assessment programme in Africa:

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Financial support for the implementation of the programme was gratefully received from:

The Global Alliance for Vaccine Initiative, through the Pediatric Bacterial Meningitis Surveillance Programme, WHO

The Government of the Kingdom of the Netherlands

The United States Agency for International Development (USAID)

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The editors are grateful for the contribution of the following individuals during the review of this document:

Centers for Disease Control and Prevention (CDC), United States of America:
Ms Cheryl Bopp
Dr Leonard Mayer
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National Institute for Communicable Diseases (NICD), National Health Laboratory Service, South Africa:
Ms Lorraine Arntzen
Ms Rita van Deventer
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Dr Anne von Gottberg
Dr Karen Keddy
Ms Rebecca Landsberg
Ms Helen Haritos

Finally, the editors would like to thank both the National Institute for Communicable Diseases, South Africa (NICD) and the Centers for Disease Control and Prevention, United States of America, (CDC) for kindly permitting the reproduction of Annexes 2-6.

Abbreviations

AFRO	WHO Regional Office for Africa
CDC	Centers for Disease Control and Prevention, USA
CSR	WHO Department of Communicable Disease Surveillance and Response
EMR	Eastern Mediterranean Region
EMRO	WHO Regional Office for the Eastern Mediterranean
EPR	WHO Department of Epidemic and Pandemic Alert and Response
EQA	External Quality Assessment
GAVI	Global Alliance for Vaccine Initiative
IDSR	Integrated Disease Surveillance and Response
IHR	International Health Regulations
IEC	International Electrotechnical Commission
ISO	International Organization for Standardization
LYO	WHO Lyon Office for National Epidemic Preparedness and Response
MoH	Ministry of Health
NHLS	National Health Laboratory Service, South Africa
NICD	National Institute for Communicable Diseases, a division of the National Health Laboratory Service, South Africa
PBM	Regional Pediatric Bacterial Meningitis Surveillance
PT	Proficiency testing
GMP	Global Malaria Programme
STB	Stop Tuberculosis Partnership
TB	Tuberculosis
USAID	United States Agency for International Development
WHO	World Health Organization
WHO CC	WHO Collaborating Centre

Glossary

Analyte — a substance that is being analyzed.
Corrective action — an exercise performed for the purpose of technical improvement.
External quality assessment (EQA) — in this document, the determination of participating laboratory performance by means of inter-laboratory comparisons.[1] Sometimes called proficiency testing.
Grade — a mark assigned by the organizer based on comparison with the expected value and taking into consideration inter-laboratory comparisons, observations, and feedback. Also called mark.
Grading area — an analytic test that the participating laboratory performs and for which the organizer assigns a grade. Also called marking area.
Mark — See grade.
Marking area — See grading area.
Organizer — agency or laboratory with responsibility for coordinating all the activities necessary for the operation of the EQA programme.
Participants — referee and participating laboratories.
Proficiency testing (PT) — see external quality assessment (EQA).
Quality assurance — the sum of all those activities in which the laboratory engages to ensure that the information generated by the laboratory is correct[2]. All those planned and systematic actions necessary to provide adequate confidence that a product, process, or service will satisfy requirements for quality[3]. A system designed to continuously improve the reliability and efficiency of laboratory services, which includes quality control, external quality assessment, and quality improvement[4].
Quality — the totality of features and characteristics of a product or service that bear on its ability to satisfy a given need[5].
Quality control — operational techniques to assure the accuracy and precision of laboratory procedures, equipment and materials.
Referee laboratory — specialized laboratory that, based on its expertise, is invited to provide technical consultation to the organizer.

[1] ISO/IEC Guide 43, part 1, 3.6:1997.
[2] Inhorn, S.L. *Quality Assurance Practices for Health Laboratories*, American Public Health Association, 1978, p. 3.
[3] ISO 8402, 23.5:1994.
[4] External Quality Assessment for AFB smear microscopy. Association of Public Health Laboratories, 2002, p. 6.
[5] Inhorn, S.L. *Quality Assurance Practices for Health Laboratories*, American Public Health Association, 1978, p. 252.

Survey— a package of challenge materials sent from the organizer to the participating laboratory in order to assess the performance of the laboratory. The survey is composed of a combination of the following materials: instructions, specimens, a clinical context for the specimens, response form, and educational resources.

1 Introduction

1.1 Background

The WHO/NICD microbiology External Quality Assessment (EQA) programme was established as a result of the Integrated Disease Surveillance and Response (IDSR) strategy;[6] a strategy adopted in 1999 by the WHO Regional Office for Africa (AFRO) to strengthen capacity for surveillance and response to priority diseases. The IDSR guidelines recommend laboratory diagnosis to confirm the nature of suspected outbreaks and guide public health response. As part of IDSR implementation, AFRO recommended the use of standard laboratory diagnostic methods for confirming priority diseases in all countries in the African Region. In an effort to monitor national laboratory capacity for implementing these standard methods, the WHO Lyon Office for National Epidemic Preparedness and Response and AFRO collaborated with the National Institute for Communicable Diseases (NICD), a division of the South African National Health Laboratory Service (NHLS), in Johannesburg, in the establishment of the WHO/NICD microbiology EQA programme.

1.2 History of the WHO/NICD microbiology EQA programme

In 2002, WHO developed the concept of a regional microbiology EQA programme for national laboratories in Africa, focusing on epidemic-prone bacterial diseases and identified NICD as the technical organizer. NCID is recognized for its technical expertise in laboratory diagnosis of priority diseases and experience in implementing national and international EQA for health laboratories in the WHO African Region.

The programme was modeled on the Canadian Microbiology Proficiency Testing (CMPT) Program,[7] organized by the Department of Pathology and Laboratory Medicine at the University of British Columbia in Vancouver, Canada. In June 2002, the CMPT Program provided technical training and consultation to NCID on EQA sample preparation and programme operations. The EQA policies and procedures were developed based on norms and standards of internationally-recognized organizations for quality issues, including the International Organization for Standardization (ISO), the International Electrotechnical Commission (IEC), the International Federation of Clinical Chemistry (IFCC), and European Eurachem[8].

[6] Centers for Disease Control and Prevention and World Health Organization. Technical Guidelines for Integrated Disease Surveillance and Response in the African Region. Atlanta, Centers for Disease Control and Prevention, 2001: 1-229.
[7] Please visit www.cmpt.ca
[8] Eurachem is a network of organizations in Europe, having the objective of establishing a system for the international traceability of chemical measurements and the promotion of good quality practices.

In July 2002, the WHO/NICD microbiology EQA programme was initiated with support from the United States Agency for International Development (USAID) and the Global Alliance for Vaccines and Immunization (GAVI). Thirty-six laboratories in 29 countries in the WHO African Region participated and were evaluated for their capacity to diagnose bacterial diseases with epidemic potential, i.e. bacterial meningitis, bacterial diarrhoeal diseases, and plague. Since then, the programme has grown significantly. As of December 2006, participation had increased to include 68 laboratories in 43 of the 46 countries in the WHO African Region, and 4 laboratories in three countries of the WHO Eastern Mediterranean Region. Moreover, the number and complexity of the assessments have increased, and in September 2005, additional diseases (malaria and tuberculosis) were assessed. Attesting to its value, the programme has served as a model for regional and national EQA within Africa and beyond.[9] The continued support and encouragement from USAID and GAVI enabled the programme to enter its fifth year in June 2006.

1.3 Purpose of this document

This document is intended to:

- describe the WHO/NICD microbiology EQA programme
- describe current policies and procedures
- provide samples of technical documents
- summarize the contents of the past surveys (July 2002–January 2006).

This document is not intended to provide results of the past surveys.

1.4 Target audience

This document is primarily intended for individuals participating in the WHO/NICD EQA programme, namely:

- laboratory managers and staff of participating and referee laboratories
- NICD technical implementation group.

It may also be useful in the training of supervisors of national public health surveillance systems and for individuals with responsibilities in the area of laboratory capacity development, such as:

- directors of national public health laboratories
- national-level health officers responsible for quality systems and laboratory strengthening
- directors of national disease prevention programmes

[9] In 2004 the WHO Eastern Mediterranean Regional Office (EMRO) began the establishment of a regional EQA. In 2006, representatives from Niger, Mali, Rwanda, Sénégal, Uganda and Zambia were trained and planned a national EQA, both patterned after the WHO/NICD EQA scheme.

- international stakeholders interested in supporting laboratory strengthening.

1.5 Dissemination and use of the document

This document is distributed to participants and stakeholders of the WHO/NICD microbiology EQA programme. It is also available as a downloadable file on the WHO web site.[10] It is intended as a practical tool to be used as a reference for the policies and procedures of the WHO/NICD microbiology EQA programme, and a model for the establishment of other EQA programmes in resource-limited countries.

[10] www.who.int

2 Quality assurance and the WHO/NICD microbiology EQA programme

2.1 Quality assurance

The implementation of quality assurance (QA) is an important activity in managing a health laboratory. QA is the sum of all the activities performed by the laboratory to provide confidence that quality objectives have been met. The quality objectives should address all elements of the laboratory and QA should involve everyone who participates in the entire process of laboratory testing. At a minimum, QA should ensure that:

- tests are performed correctly
- results are accurate, comparable, and reproducible

2.2 errors are detected and corrected to avoid adverse outcomes.External quality assessment

External Quality Assessment (EQA) is one component of quality assurance. Several definitions of EQA exist, leading to confusion. EQA can consist of:

- on-site evaluation of laboratories by standardized techniques
- panel testing, also called proficiency testing
- rechecking.[11,12]

In this document, EQA is used in the sense of proficiency testing i.e. a systematic assessment by an external organization administering surveys to participating laboratories, and the laboratories being evaluated by their responses to the surveys.

Each survey consists of specimens and a questionnaire focusing on clinical syndromes or diseases (e.g. meningitis, diarrhea, plague). The participating laboratories analyse the specimens using recommended methods, complete the questionnaire, and report back to the organizer. The organizer evaluates the results, assigns a score to each participating laboratory, and communicates the scores and explanatory comments.

The surveys are identical for all participating laboratories, and, when feasible, the specimens simulate real clinical samples. Participating laboratories are expected to process the survey specimens using the same methods which they use routinely with patient specimens. Therefore, EQA is considered as an indirect assessment of laboratory performance with clinical samples.

[11] External Quality Assessment for AFB Smear Microscopy. Association of Public Health Laboratories, 2002.
[12] Basics of Quality Assurance for Intermediate and Peripheral Laboratories, WHO/EMRO, 2002.

In the case of microbiology EQA, the responses are evaluated against a pre-determined intended response (e.g. the identity of the organism and its antimicrobial susceptibility pattern). In the case of clinical chemistry and haematology, responses are usually compared with the performance of a number of laboratories.

Many EQA programmes also provide educational resources such as technical commentaries, monographs or reference documents on selected topics.

2.3 Objectives of the WHO/NICD microbiology EQA programme

The objectives of the WHO/NICD microbiology EQA programme are intended to benefit both participating laboratories and public health programmes.

The objectives that benefit participating laboratories are those which:

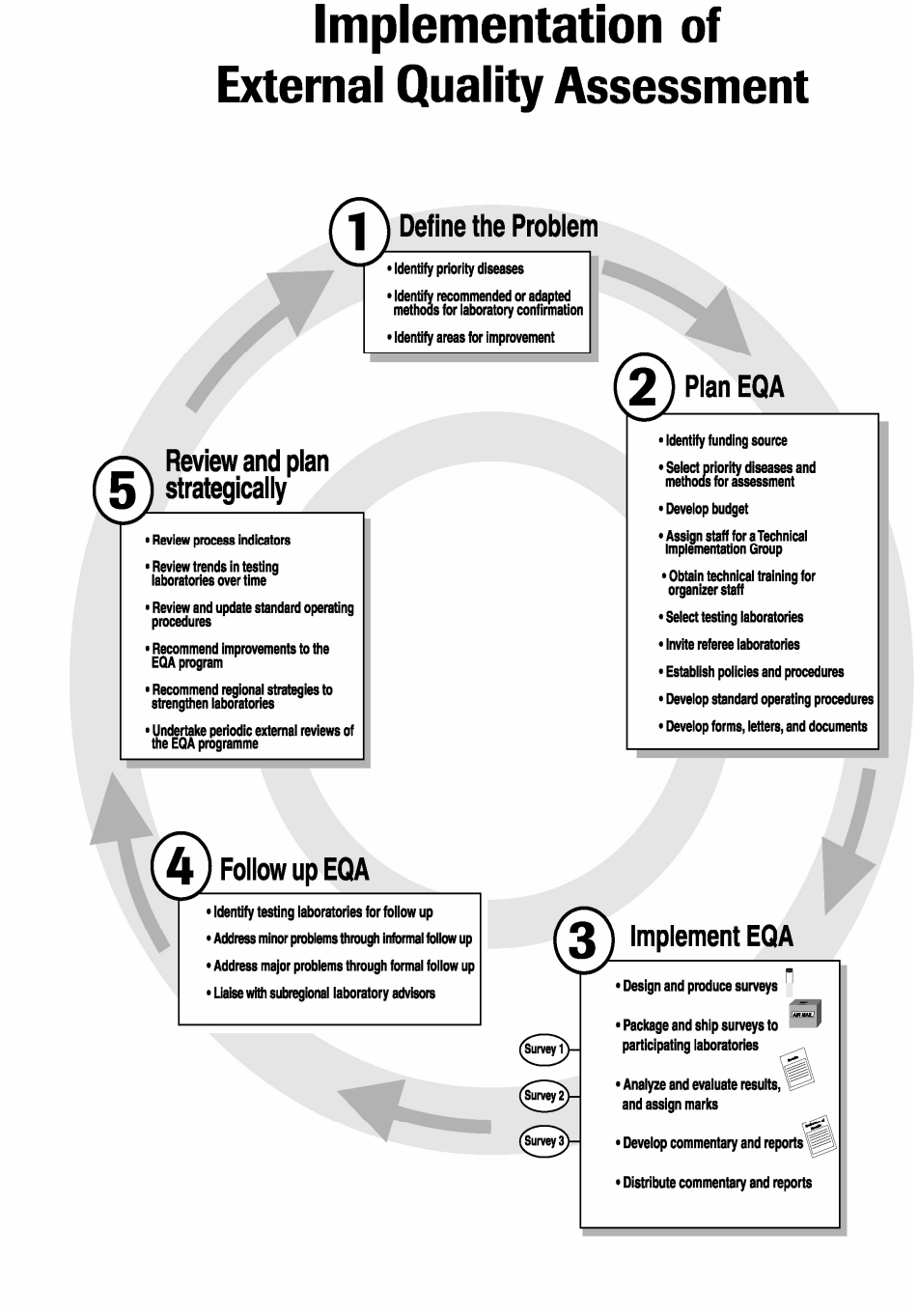
- identify and evaluate the capabilities of laboratories through an external assessment
- guide laboratories in corrective action and continuous improvement
- provide continuous education to laboratory staff on standard diagnostic methods
- raise awareness of the successes and challenges in laboratory practice
- provide information for advocacy.

The objectives that benefit public health programmes are those which:

- provide data for identifying strategies to improve laboratory competency
- guide the planning and evaluation of laboratory training
- identify laboratories of excellence
- reinforce communication networks among laboratories
- provide information to advocate for the development of laboratories
- strengthen links between WHO vertical programmes for disease prevention and control.

The implementation of these objectives involves a systematic approach that is illustrated in Figure 1.

Figure 1: Implementation of an EQA programme[13]



[13] Reproduced with the permission of CDC.

3 Clinical conditions and diagnostic tests

3.1 Clinical conditions

The clinical conditions addressed from the start of the WHO/NICD microbiology EQA programme include bacterial meningitis, bacterial diarrhoeal diseases, and plague. During Year 4 (2005–2006) tuberculosis and malaria were added. These were chosen for EQA because they feature among the priority diseases selected by the AFRO for IDSR.[14] Their priority designation is based on their inclusion in one or more of the following categories:

- diseases that are among the top causes of high morbidity and mortality in Africa (for example, malaria, diarrhoeal diseases, tuberculosis, meningococcal meningitis)
- diseases that have epidemic potential (meningococcal meningitis, cholera, plague)
- diseases for which surveillance is required internationally (plague, cholera)
- diseases for which effective control and prevention interventions exist (meningitis, diarrhoeal diseases, plague, malaria).

In addition, these conditions were targeted for EQA because laboratory testing confirms their diagnosis and guides decisions for their prevention and control. Finally, there is a lack of external quality assessment options for these diseases available to laboratories in resource-limited areas.

3.2 Diagnostic tests

The diagnostic tests evaluated through this EQA programme are those recommended by AFRO for the confirmation of suspected outbreaks if priority diseases[15] (Table 1).

[14] Centers for Disease Control and Prevention and World Health Organization. Technical Guidelines for Integrated Disease Surveillance and Response in the African Region. Atlanta, Centers for Disease Control and Prevention, 2001: 13.
[15] WHO CDC Guidelines for Integrated Disease Surveillance and Response in the African region.

Table 1. Clinical conditions and diagnostic test

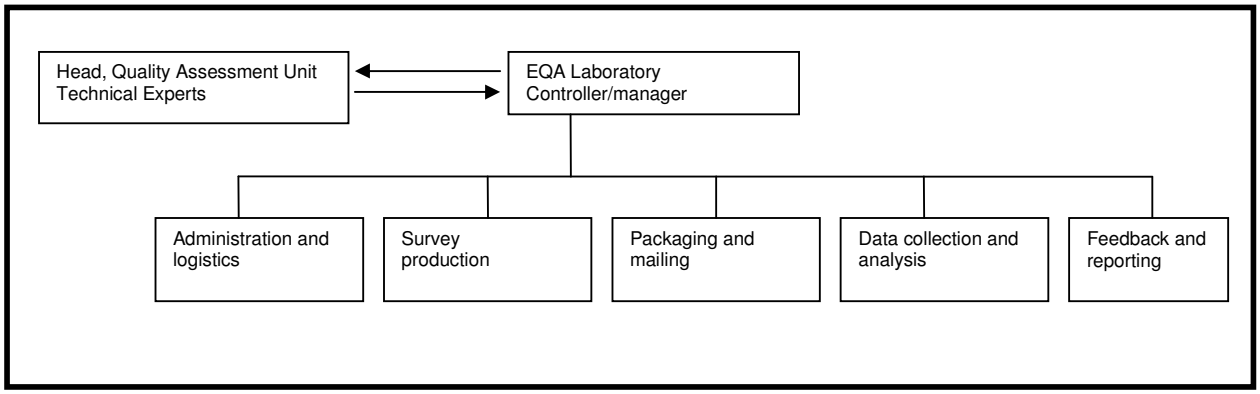
Clinical conditions (causative agents)	Diagnostic tests included in the EQA programme
Bacterial meningitis (*Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*)	Microscopy Culture and identification Antimicrobial susceptibility testing
Bacterial diarrhoeal diseases (*Salmonella* spp., *Shigella dysenteriae*, *Vibrio cholerae*)	Culture and identification Antimicrobial susceptibility testing
Plague (*Yersinia pestis*)	Microscopy. Culture and identification Antimicrobial susceptibility testing Serology Dipstick assay for F1 antigen detection
Malaria (*Plasmodium* spp. especially *Plasmodium falciparum*)	Microscopy and parasite density quantitation

4 Organization of the WHO/NICD microbiology EQA programme

4.1 Technical implementation group

The Technical Implementation Group (TIG) coordinates the operational activities of the EQA programme. This group is based in a division of the National Health Laboratory Service: the National Institute for Communicable Diseases (NICD) in Johannesburg, Republic of South Africa. Within this group, the EQA laboratory controller/manager oversees all operations in consultation with the technical experts and the Head of the Quality Assessment Unit.

Figure 2: Functions of the TIG



The responsibilities of the TIG are to:

- plan the **frequency** of surveys
- define the **type and number of specimens** to be provided in each survey
- select **quality control materials** for distribution to participating laboratories
- develop **Standard Operating Procedures** (SOPs) for all implementation operations
- define acceptable **time limits** for the transfer of surveys and exchange of information
- **produce** surveys
- **package** surveys to avoid deterioration during transportation
- **ship** surveys in compliance with national and international regulations
- define a scheme for **grading** results
- **assess** all results
- define the limits of **acceptable results**
- ensure **confidentiality** of the results

- provide **reports** of the assessment of results to participating laboratories and to the Technical Advisory Group (see below)
- guide participating laboratories in **improvements**, as needed
- participate in an **external evaluation** to ensure that their performance meets required standards
- **coordinate** with referee laboratories.

4.2 Participating laboratories

The participating laboratories are chosen by AFRO to take part in the EQA for the purpose of assessing their performance. They include laboratories designated by Ministries of Health as national public health laboratories. In addition, other major laboratories that support public health surveillance and response within the country are invited to participate. Most participating laboratories are affiliated with public hospitals, and their public health functions comprise a minor proportion of their services. Some participating laboratories are affiliated with either public health or medical research institutions.

By Year 4 of the WHO/NICD EQA, 72 laboratories were participating in the programme; 68 laboratories from 43 of the 46 WHO African Region countries[16] and four laboratories in three countries in the meningitis belt in the WHO Eastern Mediterranean Region[17] (see Annex 1).

Participation in the EQA programme is voluntary and without charge to the participating laboratories.

The responsibilities of each participating laboratory are to:

- provide appropriate **contact information** to facilitate the prompt receipt of the surveys, reports, and other communications
- **allocate duties** to all staff members who will process surveys
- **process** the surveys in the same way routine samples are handled
- **ensure** (and indicate by signing the report) that all testing of the surveys is done in the participating laboratory using the methods indicated in the report
- provide the requested information on the **methods and results**
- **report the results** of each survey to the EQA organizer within the established timeframe
- report any **problems** with the surveys to the EQA organizer
- **share the results** of the EQA with all staff members
- **collaborate** with the organizer, WHO, the health authorities and partners to address problems highlighted by EQA.

[16] Liberia, Mauritius and South Africa were not enrolled.
[17] Djibouti, Somalia, and Sudan.

4.3 Referee laboratories

Referee laboratories are specialized laboratories that are invited to participate in the EQA in their area of expertise. Some are WHO Collaborating Centres. They are selected for their internationally-recognized expertise in the laboratory diagnosis of the specified diseases. They receive surveys in their specialty, identical to those received by the participating laboratories. They conduct quality control of these specimens and their results and feedback guide the TIG in determining the limits of acceptable responses. At least two referee laboratories are used for each clinical condition in the EQA. The laboratories involved as referee laboratories since 2002 are shown in Annex 1.

4.4 Technical Advisory Group

The Technical Advisory Group (TAG) is a body of technical staff from the WHO Headquarters and AFRO with experience in laboratory development in Africa, which provides guidance to the TIG. After the completion of each survey, the TAG receives a report from the TIG, reviews the results, advises on identifying strengths and weaknesses in the participating laboratories, and makes recommendations on follow-up for laboratories in need.

The responsibilities of the TAG are to:

- **review** the results of each survey
- identify participating laboratories of **excellence**
- identify factors in participating laboratories that contribute to **unsatisfactory performance**
- identify participating laboratories in need of **follow-up interventions**
- **make recommendations** to the administration of the participating laboratories, as needed, based on EQA results
- **invite technical partners** to provide advice and consultation on the development, implementation and evaluation of the surveys.

4.5 Regional Advisory Group

The Regional Advisory Group (RAG) comprises technical partners who participate with the TIG and TAG in the annual technical review of the WHO/NICD microbiology EQA programme and in the development of the programme annual plan of action. They are invited by AFRO based on their expertise in laboratory diagnostic methods, laboratory development, and quality systems. The group is coordinated by the Regional Advisor for Laboratories in the AFRO/CSR unit.

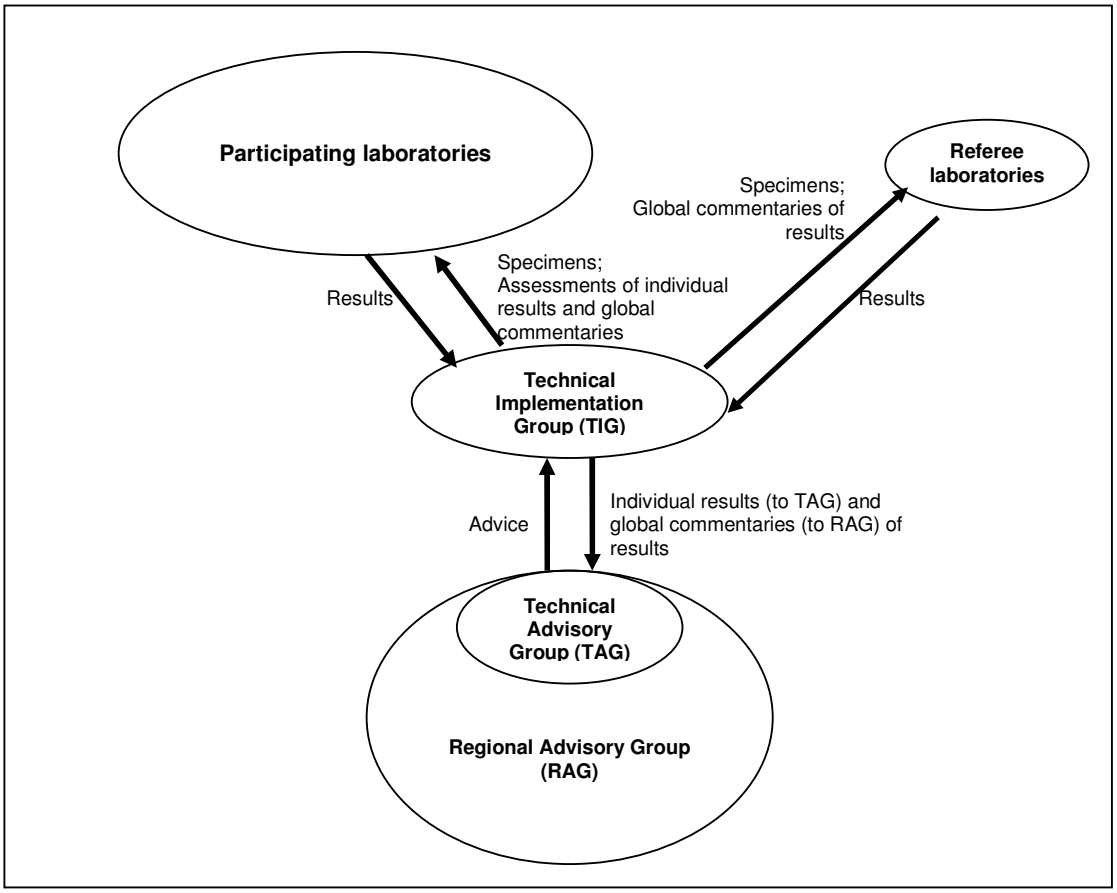
The functions of the RAG are to:

- review the technical operations and documents of the WHO/NCID microbiology EQA programme
- provide **technical assistance** for follow-up activities proposed by the TAG and implemented by the TIG after each annual review meeting
- advise the **WHO Regional Office for Africa** on strategies and interventions for laboratory strengthening
- **make recommendations** for future directions of the WHO/NICD EQA programme
- **ensure links** between vertical programmes on issues of quality assessment for laboratories in the WHO African Region.

4.6 Interactions

The Groups described above interact as shown in Figure 3.

Figure 3. Interactions between different groups in the operation of the EQA programme



5 Situation analysis

A situation analysis is an effort to collect and analyse information about the current situation of an organization or a programme. Such information on the participating laboratories can help the organizer in planning and making decisions about EQA operations. For example, information on inventory, supplies, and the condition of equipment provides insight into the technical capabilities of the laboratories. Up-to-date information on communication facilities is helpful in determining the best way for the organizer to communicate with the participants.

The TIG obtains information about laboratories participating in the WHO/NCID EQA programme from assessments conducted by the Ministries of Health and WHO and from customized questionnaires.

5.1 WHO assessments

5.1.1 WHO Regional Office for Africa assessments for IDSR implementation

AFRO recommends that countries assess the capacity of their national surveillance and laboratory systems to support IDSR. The purpose of the laboratory assessment is to determine the existing capacity of laboratories at different levels in order to provide services to support surveillance of priority diseases.

5.1.2 WHO/EPR/LYO assessments for the Laboratory Integrated Capacity Development Programme

Since 2002, the WHO Lyon Office has conducted assessments of those African laboratories participating in training cohorts of the Integrated Capacity Development Programme for Laboratory Specialists. The purpose of the assessments was to collect baseline data about the laboratory prior to its participation in the programme and to determine its capacity to perform the essential functions of national public health referral laboratories. A standard laboratory assessment tool[18] has been designed for these assessments.

5.2 NICD questionnaires

Periodically, NICD sends written questionnaires to the participating laboratories with the surveys. The questionnaires address technical and logistic issues such as testing menus, available laboratory equipment, and communication capabilities (see Annex 2 for some examples). The completed questionnaires are analysed and summarized by

[18] For information on the assessment methodology, please contact the WHO Lyon Office at oms@lyon.who.int

the TIG and presented to the RAG during the annual review meetings to guide programme improvements.

6 The EQA programme process

6.1 Enrolment of participating laboratories

The laboratories participating in the WHO/NICD EQA programme are selected by AFRO (see Section 4.2). Upon selection, NICD sends electronic and hard copies of introductory information to the participating laboratories. Simultaneously, the information is sent through AFRO to the Heads of Epidemiology and Laboratory Units at the Ministries of Health and to the WHO Country Office.

The introductory information is the first communication between the organizer on one side and the participating and referee laboratories and local stakeholders in the other side. The purpose is to communicate the objectives of EQA programme and the roles and responsibilities of those involved. The primary target audience is the directors and staff of the participating and referee laboratories.

6.2 Components of the surveys

The surveys are designed to assess the participating laboratories' capabilities in standard diagnostic methods for priority diseases. Each survey addresses each of the following: bacterial meningitis, bacterial diarrhoeal diseases, plague, malaria, and tuberculosis. Annex 3 contains a summary of surveys sent out between July 2002 and January 2006.

Each survey contains:

6.2.1 Instructions

The "Instructions" document is sent to the participating laboratories in hard copy with each survey. Its purpose is to explain what is expected from the laboratories in processing and responding to the survey. It also provides the timeframe within which the results should be submitted and when to expect feedback.

6.2.2 Specimens

The specimens represent the types of specimens that are received routinely by the participating laboratories. They may be actual clinical samples, lyophilized strains, or simulated specimens. Simulated specimens are specifically prepared to mimic clinical samples.

6.2.3 Report form

The report form describes the clinical context for the survey specimens and provides a standardized format for the results. It is sent in hard copy with each survey and can also be downloaded from the WHO Resource Centre for Public Health Laboratories.[19]

Participating laboratories return the completed forms by e-mail or fax.

The report form contains two parts:

Clinical context

The clinical context describes the simulated case history and the origin of the specimens. Each case history is appropriate for Africa. For example, a bacterial meningitis survey may describe a clinical case of meningitis (caused by *Neisseria meningitidis* serogroup W135) consistent with recent outbreaks in West Africa. Similarly, a survey on bacterial diarrhoeal diseases may be based on a case of shigellosis (caused by *Shigella dysenteriae* type 1) such as those which have occurred in central Africa. The clinical context may also contain relevant information about the specimens, such as any results of other tests, handling details, etc.

Questionnaire

The questionnaire is the standardized format for the laboratory to use when recording results of the survey. It may also include questions about the testing algorithm and the methodology used in the laboratory.

6.3 Production and distribution of surveys

6.3.1 Preparation of surveys

Approximately three weeks prior to the survey date, the organizer begins preparing the clinical case histories and the simulated specimens according to the Standard Operating Procedures (SOPs) shown in Annex 4.

NB. If it is necessary to collect specimens from individuals for use in EQA, informed consent is obtained in full compliance with the regulations of the Internal Review Board and the Ethics Committee of NICD and the University of the Witwatersrand, South Africa.

[19] The interactive resource centre for public health laboratories was established by the WHO Lyon Office in May 2005 and is available at www.who.int/labresources .

6.3.2 Packaging

The surveys are packaged for shipment, according to SOPs, to comply with the following regulations:

- Shipping Guidelines for Infectious Substances developed by the International Air Transport Association (IATA);
- UN Recommendations on the Transport of Dangerous Substances (Class 6).[20]

6.3.3 Distribution

Surveys are distributed on the basis of the routine services provided by the participating laboratory. For example, all participating laboratories receive surveys for bacterial meningitis and diarrhoeal diseases, because they all routinely provide such testing. However, only a subset of laboratories receives plague surveys as not all laboratories offer plague diagnosis.

6.3.4 Shipping and tracking

Shipping of surveys is done by rapid air courier. This method provides fast, reliable, and traceable services in areas without effective postal services and grant a tracking system for the sender.

The rapid air courier delivers the surveys according to the courier's corporate policies for non-dangerous or dangerous goods, as the case may be; door-to-door delivery is given priority, wherever possible. If the policy allows for delivery only to the nearest airport, the EQA organizer provides specific instructions to the air courier for proper storage of the shipment and for telephone or e-mail communication with the recipient to arrange for pick-up.

The shipped surveys are tracked by the TIG using the tracking service on the web site of the rapid air courier. The date of delivery to the participating laboratory provided by the tracking system is used in the calculation of the response time.

6.4 Timeframes

6.4.1 Survey date

Survey date refers to the day when the specimens are sent out by the organizer. The number of surveys sent out per year is based on the available budget, the time necessary for implementation of each survey, and the number of surveys recognized as

[20] According to current regulations, pure cultures of *V. cholerae, S. dysenteriae* 1, and *Y. pestis* are considered "Infectious Substances, Category A".

necessary to maintain an effective EQA. It has been established that the distribution of three surveys per year meets these criteria.

6.4.2 Reminder e-mail date

A reminder e-mail is a message sent to each participating laboratory that has not yet submitted results for the current survey. Its purpose is to remind the participating laboratory to submit its report form before the closing date. The reminder e-mail date is four days prior to the closing date.

6.4.3 Closing date

The closing date is the date after which the results from the participating laboratories will be considered late and not acceptable.

The closing date is 15 working days after the last participating laboratory receives the survey, according to tracking data of the rapid air courier service. This interval is based on the period of time considered reasonable for the participating laboratories to analyse the specimens.

6.5 Evaluation of survey results

The TIG receives the report forms from the participating and referee laboratories, reviews them and assigns each a mark or grade.

The assessment takes into account the following information:

- expected results on the required tests
- results, observations and feedback from referee laboratories
- results, observations and feedback from the participating laboratories.

All these sources are considered because unexpected events may prevent laboratories from achieving the expected result. While quality control practices during production can prevent many problems, adverse events may occasionally remain undetected until the specimens are analysed by another laboratory. Moreover, unpredictable shipping conditions may jeopardize the viability of the pathogens or the stability of the specimens.

The evaluation process is accomplished by the TIG during a formal survey review meeting.

6.5.1 Marking (or grading) areas

Marking areas are sections of the survey results to which the TIG assigns marks. Each grading area corresponds to a critical decision point or an analytical test that the participating laboratory performed. Depending upon the specimens in the survey and the tests conducted, the grading areas may include one or more of the following:

- microscopy
- culture and identification
- serotyping
- antimicrobial selection
- antimicrobial results and reporting
- rapid test results (plague F1 antigen)
- malaria parasite density quantitation.

For each area, the participating laboratory receives a separate grade. The total score for the survey is the sum of all scores of the marking areas. Partial evaluation, i.e. withdrawal of a marking area from consideration without affecting the overall score, is done only in exceptional circumstances.

6.5.2 Marks (grades)

The TIG attempts to mark all surveys consistently and uses the following scheme.[21]

Score	Description	Criteria for assignment of score
4	Full value	Accepted by the committee as the correct answer either in terms of current nomenclature or in terms of appropriate clinical relevance.
3	Essentially correct or acceptable	A nomenclature or antimicrobial susceptibility error, generally at the species level, not technically correct but would have little or no clinical impact. A deviation from what is considered the most clinically relevant result, but one which would pose little difficulty in interpretation of the sample's report.
Separator		**To augment the difference between the two groups**
1	Incorrect or unacceptable	A nomenclature error that would be wrong at the species level, but by reporting may have an impact on the clinical interpretation and potentially a treatment error. A major antimicrobial susceptibility error. A clinically relevant result that could lead to a diagnosis or treatment error.
0	Very incorrect or very unacceptable	A nomenclature error that would be wrong at the genus and species level or a major antimicrobial susceptibility error that could result in a significant interpretation or treatment error. A clinically relevant result that could lead to a major diagnostic or treatment error.

[21] The grading scheme was adopted from the methodology used by the Canadian Microbiology Proficiency Testing Program.

The marking scheme is established and the results of each participating laboratory are scored in accordance with it. Individual confidential reports of the scores and a global commentary (see below) are sent to all participating laboratories. The referee laboratories receive only the global commentary. The TAG receives the global commentary and a confidential survey data sheet (see below).

6.6 Documentation of results and feedback to participating laboratories

6.6.1 Confidentiality of reports

Results from participating laboratories are treated as confidential. Reports identifying the participating laboratories are not shared outside the TAG and this group respects the confidentiality of the participating laboratories when they make use of these reports.

6.6.2 WHO/NICD EQA Confidential Report

The WHO/NICD EQA Confidential Report is an individual feedback report customized for each laboratory so that it contains only that laboratory's survey scores. It also includes an explanation of the grading criteria and technical commentary on any discrepancies compared with the acceptable results. The purpose of this report is to provide individualized feedback on the laboratory's performance in a survey and to offer recommendations for improvement. The recipient laboratory can use the information as a review of its methods and a guide for improvements.

The report is sent by e-mail two weeks after the closing date, and in hard copy with the next survey.

6.6.3 Corrective Action Sheet

The Corrective Action Sheet is targeted to specific participating laboratories. It contains a technical improvement exercise linked to the problems identified through the laboratory's performance in EQA. The exercise aims to guide the laboratory staff in identifying the cause of the unacceptable results and making changes to correct the problem.

6.6.4 WHO/NICD EQA Global Commentary

The WHO/NICD EQA Global Commentary is a comprehensive report of a survey which is intended for all participating and referee laboratories and for the RAG. This document displays the correct or acceptable responses, the scores from all the participating laboratories (without identifiers), and commentary describing the minor and major problems encountered.

The purpose is to show the inter-laboratory comparisons and to highlight problems that may have interfered with acceptable performance. Laboratories can use this report to understand problems experienced by themselves and their peers.

The Global Commentary is sent by e-mail five weeks after the closing date, and in hard copy with the next shipment.

6.6.5 Confidential Survey Data

The Confidential Survey Data is a spreadsheet containing all the results and scores from a single survey. This includes the correct or expected results, the results from all participating laboratories, and the results from the referee laboratories. All laboratories are identified in this document. The purpose is to show inter-laboratory comparisons and to highlight problems that might have interfered with acceptable performance.

The target audience is the TAG, which uses these data to assess performance, identify problems, and recommend follow-up. The Confidential Survey Data spreadsheet is sent to the TAG by e-mail four weeks after the closing date.

6.6.6 WHO/NICD EQA Yearly Report

The WHO/NICD EQA Yearly Report is a summary of all the scores of all the surveys in a single year. This includes the correct or expected scores and the scores of all participating and referee laboratories. No laboratories are identified in this document. The target audience includes all participating and referee laboratories, local and international stakeholders, the TAG and the RAG.

The Yearly Report is distributed by e-mail four weeks after the closing date of the last survey of the year and as a hard copy during the RAG annual review meeting.

6.7 Data management

Data from EQA results are entered into Microsoft Excel® and Access® electronic databases and backed-up weekly. All results, data analysis, evaluations, and reports are archived and will be readily accessible for five years.

7 Documents

Standardized documents facilitate the operation of the EQA. Some documents address technical procedures and aim to promote quality in the technical operations and management of EQA activities. Other documents are used for communications between the TIG, participating and referee laboratories, advisory groups, and stakeholders. These documents aim to promote a clear understanding of the goals, objectives, and findings of the EQA programme.

All documents drafted by the TIG and are reviewed and approved by the RAG.

7.1 Technical documents

7.1.1 Standard Operating Procedures

An SOP is a written procedure that all staff must follow when performing a routine laboratory task. Each SOP details the steps of the task in the order in which they should be performed. The use of written SOPs assures quality and reliability in the performance of diagnostic tests and in the development of the EQA surveys. Written protocols also provide a basis for troubleshooting any problems that may arise.
Annex 4 shows SOPs developed for the WHO/NICD microbiology EQA programme.

7.1.2 Technical monographs

The monographs are intended to provide theoretical and practical information to enhance knowledge and technical competence of staff in the participating laboratories. They include NICD and WHO documents, and can be used by the participating laboratories for in-service training and review. They are distributed (in hard copy) with the surveys and are also available from the Internet WHO Resource Centre.

7.2 Documents for communication

These documents enable the communication of information, results, feedback, and reports on the EQA programme. They include introductory information, instructions, report forms (with clinical context and questionnaire), forms for feedback to participating laboratories and to the TAG and RAG, the Corrective Action Sheet, and the WHO/NICD EQA Global Commentary. Their use is described in Section 6 and templates are provided in Annex 5. They are produced in French and English, and when possible, in Portuguese and the translations are distributed according to each country's needs.

8 Monitoring and evaluation of the WHO/NICD microbiology EQA programme

8.1 Monitoring process indicators

The TIG monitors process indicators for each survey. These indicators include:

- the percentage of participating laboratories submitting a response
- the average reporting time in days
- the mean overall score
- the percentage of participating laboratories with acceptable scores in each grading area.

These data are available on a CD-ROM produced after each annual review meeting and distributed to the RAG members. They are also available in the WHO/NICD EQA Yearly Report.

8.2 External evaluation

In 2006, the NICD EQA unit has started an accreditation process with the South African National Accreditation System (SANAS) to be accredited to the ISO 43-1:1997 standard, to ensure compliance with internationally recognized standards. It is expected that the impact of the WHO/NICD EQA programme on the participating laboratories will be evaluated in 2007 by an external recognized expert of external quality assessment.

9 Follow-up

Follow-up with participating laboratories ranges from informal consultation requiring minimal resources, to formal assessments and training activities that entail substantial commitments of resources and the engagement of external partners.

9.1 Informal follow-up

Informal follow-up consists of e-mail or telephone consultation by between the TIG and a participating laboratory. Any participating laboratory is entitled to informal follow-up upon request.

9.2 Formal follow-up

Formal follow-up consists of activities customized to the needs of a participating laboratory. It may include a visit to the laboratory by a member of the TIG and other technical experts to conduct a situation analysis and/or focused training. A site visit offers the opportunity for direct observation of the work environment, training of staff, and advocacy with the ministry of health.

9.3 Web-based follow-up

Follow-up is also available via an interactive Internet-based resource centre.[22] A specific section has been created for all participants of the regional microbiology EQA programmes sponsored by the WHO Lyon office which allows:

- the download of EQA general documentation
- the download of specific documentation for the WHO microbiology EQA programmes
- the download of the report forms to be completed
- a discussion forum with the other participants.

[22] www.who.int/labresources

10 Perspectives and challenges

10.1 Perspectives

The WHO/NICD microbiology EQA programme serves as an important tool for countries in fulfilling their national and international roles in health security.

Annex 1 of the International Health Regulations (2005) states that States Parties shall have the capacity to provide support to the response to a public health emergency of international concern (PHEIC)

> "through specialized staff, laboratory analysis of samples (domestically or through collaborating centres) and logistical assistance (e.g. equipment, supplies and transport)."

The WHO/NICD microbiology EQA programme provides a means to ensure that this laboratory analysis is of high quality. It also allows the documentation of laboratory capacity at national-level and a guide to improvements. In addition, countries recognize the need to establish national networks of regional/provincial and district laboratories to fulfill public health functions. Ongoing laboratory development and evaluation will be important in this effort.

The WHO/NICD microbiology EQA programme described in this document can be adapted by Ministries of Health to meet their needs to assess national laboratories and networks. Many resources are available to laboratory specialists tasked with such an adaptation:

- all of the templates and SOPs presented in this document can be freely utilized;
- the viable specimens received by the participating laboratories can be subcultured and distributed to other laboratories (in accordance with the safe handling and transport of specimens and national recommendations);
- training and reference materials and monographs used in workshops conducted by WHO and linked to this EQA programme or distributed through this programme are available from participating laboratories;
- materials and tools developed by WHO Lyon Office for public health laboratories which can be shared upon request[23].

10.2 Challenges

Sustainable financial support and political commitment are ongoing challenges for the WHO/NICD microbiology EQA programme. These essential inputs can impact three major elements.

[23] oms@lyon.who.int

10.2.1 The scope of the programme

The core programme, supported by USAID, focuses on the bacterial diarrhoeal diseases, meningitis and plague. In 2005, with the support of the Government of the Netherlands, malaria and tuberculosis microscopy EQA was added to the programme. Additional support is required to sustain the programme at its current level and expand it to other epidemic-prone diseases or other laboratory medicine components. Collaboration across disease-specific programmes in WHO such as the Stop TB Partnership and the Global Malaria Programme should be encouraged as laboratory quality is a common concern for all these programmes.

10.2.2 Follow-up activities for laboratories in need

Activities described in Section 9 provide opportunities to pinpoint problems in laboratories with difficulties and provide targeted assistance. These are critical steps in improving the performance of laboratories. Effective follow-up requires significant financial support, particularly for site visits to selected laboratories. Equally important is the political commitment from the health authorities to implement any recommendations prompted by follow-up activities.

10.2.3 Essential human and material resources

Performance of participating laboratories depends not only on technical expertise, but also on adequate staffing, equipment, supplies and reagents. Political commitment within the national governments and Ministries of Health is essential to find the means to provide the resources needed by laboratories to fulfill their critical role in public health.

11 Bibliography

Selection, use, and interpretation of proficiency testing schemes for laboratories, 1st ed. Eurachem Nederland, 2000:1–47 (http://www.eurachem.org/guides/ptguide2000.pdf , accessed 8 May 2007).

International Federation of Clinical Chemistry. *Fundamentals of External Quality Assessment (EQA). Guidelines for improving analytical quality by establishing and managing EQA schemes* (http://www.ifcc.org accessed 8 May 2007).

International Laboratory Accreditation Cooperation. *Guidelines for the requirements of the competence for providers of proficiency testing schemes*; 2000 (http://www.ilac.org/documents/ILAC_G13_2000_guidelines_for_the_requirements_for_the_competence_of_providers_of_proficiency_testing_schemes.pdf accessed 8 May 2007).

International Organization for Standardization and International Electrotechnical Commission. *Proficiency testing by interlaboratory comparisons – Part 1: Development and operation of proficiency testing*; 1997; ISO/IEC Guide 43-1:1–16.

International Organization for Standardization and International Electrotechnical Commission. *Proficiency testing by interlaboratory comparisons – Part 2: selection and use of proficiency testing schemes by laboratory accreditation bodies*; 1997; ISO/IEC Guide 43-2:1–3.

UNAIDS. *Guidelines for Organizing National External Quality Assessment Schemes for HIV Serological Testing*; 1996; UNAIDS/96.5:1–35.

World Health Organization. *Requirements and guidance for external quality assessment schemes for health laboratories*; 1999; WHO/DIL/LAB/99.2:1–65.

Association of Public Health Laboratories. *External Quality Assessment for AFB Smear Microscopy*, 2002 (http://www.aphl.org/programs/infectious_diseases/EQA.cfm accessed 8 May 2007).

ANNEX 1

Organizational groups and participating laboratories for the WHO/NICD microbiology EQA programme

1.1 Technical implementation group (TIG)

Name	Role in Technical Implementation Group
Dr Kerrigan McCarthy	Head, External Quality Assessment Unit
Ms Vivian Fensham	EQA Laboratory Controller/Manager
Prof John Frean	Deputy Director, NICD
Dr Anne von Gottberg	Technical expert in meningitis
Dr Karen Keddy	Technical expert in bacterial diarrhoeal diseases
Ms Lorraine Arntzen	Technical expert in plague
Ms Leigh Dini	Technical expert in malaria
Ms Linda de Gouveia	Laboratory technologist, meningitis
Ms Helen Haritos	Laboratory technologist, EQA Programme

1.2 Participating laboratories (Year 4, 2005-2006)

Country	Laboratory	Receives plague specimens	Language*
Algeria	Faculté de Médecine d'Oran, Centre Hospitalier d'Oran, Service de Bactériologie, HAT ESSALNO no. 219 Oran	No	F
Angola	Nacional do Salude Publica, Luanda	Yes	P
Benin	Laboratoire National de Santé Publique 01 BP 418, Cotonou	No	F

Benin	Centre National Hospitalier et Universitaire de COTONOU, 01 BP 386, Cotonou	No	F
Botswana	National Public Health Reference Laboratory, Plot #5353 Ext 10, Church Road, Gaborone	Yes	E
Burkina Faso	Bureau Régional OMS pour l'Afrique – Centre de Surveillance Pluri-Pathologique (CSPP/MDSC), Avenue Naba Zombre N:1473 -01 BP 549 Ouagadougou 01	No	F
Burkina Faso	Laboratoire de Bactériologie, (Hôpital Yalgado Ouedraogo) CHN-YO 03, BP 022, Ouagadougou 03	No	F
Burkina Faso	Laboratoire de Bactériologie-Virologie, Hôpital Pédiatrique Charles de Gaulles, Ouagadougou	No	F
Burkina Faso	Laboratoire National de Santé Publique, Ouagadougou	No	F
Burundi	Laboratoire National de Référence, CHU de Bujumbura, BP 2210, Bujumbura	No	F
Cameroon	Centre Pasteur du Cameroun, B.P. 1274 Yaoundé	No	F
Cameroon	Laboratoire de bactériologie, Centre Pasteur du Cameroun, Garoua	No	F
Cape Verde	Laboratorio Nacional de Referencia/ Laboratorio Hospital Agostinho Neto Praia, LP 112	No	P
Central African Republic	Laboratoire National de Santé Publique Bangui	No	F
Chad	Hôpital Général de Référence BP130 N'Djamena	No	F
Comoros	Laboratoire Hôpital EL-Maarouf B. P : 17 Moroni	No	F
Congo	Laboratoire National de Santé Publique, Cité Louis Pasteur, Avenue du Général de Gaulle, BP 120 Brazzaville	No	F
Côte d' Ivoire	CHU Yopougon BP 632, Abidjan 21	No	F

Democratic Republic of the Congo	Laboratoire Provincial de Lubumbashi, Avenue Likasi N° 491, Ville de Lubumbashi, Province du Katanga	Yes	F
Democratic Republic of the Congo	Institut National de Recherche Bio-Médicales, BP 1197, Avenue de la Démocratie, Kinshasa-Gombe	Yes	F
Djibouti	Hôpital Général Peltier, BP 1323, Djibouti	No	F
Djibouti	Office de Protection Sociale, Ministère du Travail, BP 21696, Djibouti	No	F
Equatorial Guinea	INSESO Laboratory (Instituto de Seguridad Social), Hopital de Malabo, Malabo,Bioko Island	No	F
Eritrea	National Health Laboratory, Denden Street 83, Asmara	No	E
Ethiopia	Ethiopian Health and Nutrition Research Institute, Addis Ababa	No	E
Ethiopia	Tikuer Anbessa Hospital, Cherchile Road, Addis Ababa	No	E
Gabon	National Reference Laboratory, Faculté de Médecine de Libreville, University Teaching Hospital, Libreville	No	F
Gambia	National Health Laboratories, Royal Victoria Hospital, Independence Drive, Banjul	No	E
Ghana	Public Health and Reference Laboratory, Health Laboratory Services, Box 300, Korlebu Hospital, Accra	No	E
Ghana	U.G.M.S. Department of Microbiology Accra	No	E
Ghana	Laboratory Microbiology Department, Komfo Anokye Teaching hospital (KATH), Kumasi, P.O.Box 1934	No	E
Guinea	Laboratoire National de Santé Publique, Division de la Prévention, BP 3820, Conakry	No	F
Guinea	Laboratoire de Bactériologie, CHU DONKA, Conakry	No	F

Guinea-Bissau	Laboratoire National de Santé Publique, BP 50, Bissau- MINISAP, Avenue Unité Africaine	No	P
Kenya	National Public Health Laboratory Service, P.O. Box 20750, Nairobi	Yes	E
Kenya	Microbiology Laboratory, Department of Laboratory Medicine, Kenyatta National Hospital, Nairobi	No	E
Lesotho	Ministry of Health and Social Welfare P.O. Box 9560, Maseru 100	Yes	E
Madagascar	Laboratoire de Bactériologie du Centre Hospitalier d'Antananarivo - HRJA, BP 4150, Antananarivo 101	Yes	F
Malawi	Public Health Laboratory, Community of Health Sciences, Ministry of Health, Private Bag 65, Lilongwe	Yes	E
Malawi	Microbiology laboratory, Queen Elizabeth Central Hospital, Blantyre	Yes	E
Malawi	OECH Laboratory, Malaria Research and Wellcome Trust Centre, Microbiology Laboratory, Blantyre	No	E
Mali	Institut National de Recherche en Santé Publique, (INRSP), BP1771, Bamako	No	F
Mauritania	Centre National d'Hygiène, Hôpital National de Nouakchott, BP 695, Nouakchott	No	F
Mauritania	Laboratoire de Biologie Médicale, Centre Hospitalier de Nouackchott	No	F
Mozambique	Faculty of Medicine Eduardo Nondlane University, Av Salvador Allende, no 702, Maputo	Yes	P
Mozambique	Hospital Centre de Maputo, Avenida Agostinho Neto, Maputo	No	P
Namibia	Namibia Institute of Pathology, Ooievaart Road, Windhoek	No	E
Namibia	Namibia Institute of Pathology, Oshakati State Hospital	Yes	E

Niger	CERMES - BP 10887 Niamey	No	F
Niger	Hôpital National, BP238 Niamey	No	F
Nigeria	Central Public Health Laboratory, 9 Muritala Mohammed Way, Yaba, Lagos	No	E
Rwanda	Laboratoire National de Référence et de Santé Publique, BP 84, Boulevard de la Révolution, Kigali	No	F
Rwanda	Bacteriology Laboratoire, CHU de Butare	No	F
Rwanda	Laboratoire de Biologie Médicale, Centre Hospitalier de Kigali, (CHK), Kigali	No	F
Sao Tome and Principe	Centre Hospitalier de Tome	No	P
Senegal	Laboratoire de Bactériologie-Virologie, CHU Fann, BP 288, Dakar-Fann-Sénégal, Avenue Cheick Anta Diop, Dakar	No	F
Senegal	Hôpital d'Enfants, Albert ROYER, CHU DAKAR FANN, BP 5035, Dakar	No	F
Senegal	Laboratoire de Bactériologie, CHU Aristide Le Dantec, Dakar	No	F
Seychelles	National Public Health Laboratory, Ministry of Health, P.O.Box 52, Mahe	No	E
Sierra Leone	Central Referral Laboratory, Connaught Hospital, Freetown	No	E
Somalia	c/o WHO Representative / Somalia, Hargeiza office	No	E
Sudan	National Health Laboratories, P.O. Box 287, Khartoum	No	E
Swaziland	National Reference Laboratory, Mbabane Government Hospital, Mbabane	Yes	E
Togo	Institut National d'Hygiène, Ministère de La Santé, BP 1396, Lomé	No	F

Uganda	Central Public Health Lab, P.O. Box 2210, Kampala	Yes	E
Uganda	Microbiology Department, Mulago Hospital, Kampala	No	E
Uganda	St Mary's Hospital, Lacor, P.O. Box 180, Gulu	No	E
United Republic of Tanzania	Dept of Microbiology, Muhimbili Medical Centre, PO Box 65000, Dar es Salaam	Yes	E
United Republic of Tanzania	Pathology laboratory, Manzi Mmojo Hospital, Stonetown, Zanzibar	No	E
Zambia	Tropical Diseases Research Centre, 7th Floor, Ndola Central Hospital, Nkana Road, Ndola	Yes	E
Zambia	Dept of Pathology and Microbiology, University Teaching Hospital, P/B RWIX, Lusaka	No	E
Zimbabwe	National Microbiology Reference Laboratory, Harare Central Hospital, Southerton	Yes	E

* Language of documents supplied: E, English; F, French; P, Portuguese

1.3 Referee laboratories

Institut Pasteur de Bangui BP 923, Bangui Central African Republic
Unité du méningocoque, IMTSSA Parc du Pharo, BP 46 13998 Marseille armées France
Laboratoire de Biologie Clinique, Hôpital des Armées Laveran 13013 Marseille France
Centre National de Référence des *Yersinia,* Centre Collaborateur de l'OMS Institut Pasteur 28 rue du Dr Roux, 75724, Paris cedex 15 France

Institut Pasteur de Madagascar BP 1274, Antananarivo 101 Madagascar
Parasitology Laboratory Research Institute for Tropical Medicine Filinvest Corporate City Compound Alabang, Muntinlupa City Philippines
WHO Collaborating Centre for Reference and Research on Meningococci Dept of Bacteriology, National Institute of Public Health Oslo Norway
Microbiology Laboratory, National Health Laboratory Service Johannesburg Hospital Parktown, Johannesburg South Africa
C18 Microbiology Laboratory, National Health Laboratory Service Groote Schuur Hospital Observatory Cape Town, 7925 South Africa
Hospital for Tropical Diseases Department of Clinical Parasitology Mortimer Market Centre, Capper Street, London WC1E 3BG United Kingdom
Meningitis Laboratory, Meningitis and Vaccine Preventable Diseases Branch Division of Bacterial and Mycotic Diseases Centers for Disease Control and Prevention 1600 Clifton Road, Atlanta, GA 30333 USA
Epidemic Investigations and Surveillance Laboratory Foodborne and Diarrheal Diseases Branch Division of Bacterial and Mycotic Diseases Centers for Disease Control and Prevention 1600 Clifton Road, MS CO3, Atlanta GA 30333 USA
Diagnostic and Reference Laboratory, Bacterial Zoonoses Branch, Division of Vector-Borne Infectious Diseases Centers for Disease Control and Prevention Rampart Road, Fort Collins, CO 80521 USA

1.4 Technical advisory group (TAG)

Name	Title and contact information
Dr Bréhima Koumaré	Director WHO Multi-Disease Surveillance Centre Ouagadougou Burkina Faso
Dr Antoine Pierson (replaced by Dr Sébastien Cognat in October 2005)	Medical Officer Laboratory Strengthening Team WHO Office in Lyon France
Technical Implementation Group (See 1.1)	NICD Johannesburg, South Africa
Dr Thomas Aisu	Subregional Laboratory Advisor, WHO Regional Office for Africa, Great Lakes and Horn of Africa Region Kampala, Uganda
Dr Jean-Bosco Ndihokubwayo	Regional Advisor for Laboratories WHO Regional Office for Africa CSR/LAB Harare, Zimbabwe
Dr Bekithemba Mhlanga	PBM Network Coordinator WHO Regional Office for Africa VPD/LAB Harare, Zimbabwe

ANNEX 2

Questionnaires sent to participating laboratories

2.1 Inclusion of other disciplines in EQA surveys (July 2002)

Dear Laboratorian
The NHLS and the WHO Regional Office for Africa is considering expanding the EQA programme to include specimens for tuberculosis microscopy and malaria diagnosis. Could you please answer the following questions.

1. Does your laboratory offer diagnostic testing for **tuberculosis**? Yes ☐ No ☐

2. If yes, which of the following tests does your laboratory perform?
   a. Microscopy (Ziehl–Neelsen staining) Yes ☐ No ☐
   b. Microscopy (Auramine O staining) Yes ☐ No ☐
   c. Culture (on Lowenstein Jensen /other medium) Yes ☐ No ☐
   d. Culture (automated) Yes ☐ No ☐
   e. Susceptibility testing (direct proportion method) Yes ☐ No ☐
   f. Susceptibility testing (automated) Yes ☐ No ☐

3. Does your laboratory participate in an EQA programme for the following tests?
   a. TB microscopy Yes ☐ No ☐
   b. TB culture Yes ☐ No ☐
   c. TB susceptibility testing Yes ☐ No ☐

4. If the answer to any of the above questions is yes, which organization provides the TB EQA material?

5. Does your laboratory offer diagnostic testing for **malaria**? Yes ☐ No ☐

6. If yes, which of the following tests does your laboratory perform?
   a. Thick smear microscopy Yes ☐ No ☐
   b. Thin smear microscopy Yes ☐ No ☐
   c. Malaria speciation Yes ☐ No ☐
   d. Quantitation of parasitaemia Yes ☐ No ☐
   e. Malaria rapid antigen detection Yes ☐ No ☐

7. Does your laboratory participate in an EQA programme for the following tests?
   a. Malaria microscopy Yes ☐ No ☐
   b. Malaria antigen detection Yes ☐ No ☐

8. If the answer to any of the above questions is yes, which organization provides the malaria EQA material?

9. Would your laboratory be interested in participating in an EQA programme for diagnostic testing for tuberculosis or malaria?

   Yes ☐ No ☐

Thank you so much for your assistance in completing this questionnaire.
Regards, the NHLS EQA team.

2.2 Questionnaire: Evaluation of the Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World (June 2005)

Dear Colleague,

Enclosed you will find a six-page survey, which is an evaluation of the "Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World" (see picture below). We are trying to learn about the laboratories that have received this manual and whether people are using the manual for their laboratory work. We want to find out how useful the manual is for laboratories and how we can improve future manuals.

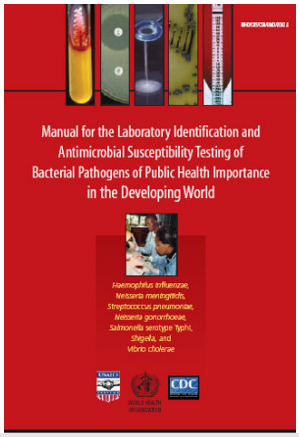
We prefer that this questionnaire be completed by someone who spends at least 30 hours per week working in your laboratory, such as a microbiologist, a lab technologist or a lab technician. If you do not participate in bench work at least 30 hours per week, please give the attached questionnaire to someone in your laboratory who does.

The opinions of the people who work in your laboratory and use this manual are very important to help us understand the good and bad things about the manual. Please be very honest when you answer the questions on the survey. Even if what you have to say is not good – that's still important for us to hear! We will keep your responses totally confidential. This means that when we discuss the findings of our evaluation, we will not share your name or the name of your laboratory.

We very much appreciate your participation and feedback.

Thank you,

Heidi Brown
Carolyn Greene, MD
Centers for Disease Control and Prevention (CDC)
Atlanta, GA 30333



THIS SURVEY SHOULD BE COMPLETED BY A FULL-TIME LABORATORIAN. IF YOU DO NOT WORK AT LEAST 30 HOURS PER WEEK IN THE LAB, PLEASE GIVE THIS SURVEY TO SOMEONE WHO SPENDS MORE TIME IN THE LAB AND ASK HIM OR HER TO COMPLETE IT.
THANK YOU VERY MUCH FOR YOUR TIME!

Today's date: ___/___/___ (dd/mm/yy)
Your name:_____
Age:_____
Your title (microbiologist, lab technician, etc.):_____
Name of laboratory where you work: _____
Country: _____

About You:

1. How many years of training did you complete before becoming a laboratorian/scientist? **(Please check only 1)**
☐ Did not complete secondary school ☐ Completed secondary school
☐ Completed 1 year of additional training ☐ Completed 2 years of additional training
☐ Completed 3 years of additional training ☐ Completed >3 years of additional training
☐ Something else? (please tell us)_____

2. For how many years have you been working as a laboratorian/scientist? **(Please check only 1)**
☐ Less than 1 year ☐ 1- 2 years ☐ 3-5 years
☐ More than 5 years

About Your Laboratory Resources:

3. Does your laboratory have access to the following resources on a daily basis?

Water purified by a filter system or a distillation apparatus	Yes	No	Unsure
Stable source of electricity	Yes	No	Unsure
Computer	Yes	No	Unsure
Internet	Yes	No	Unsure
E-mail access	Yes	No	Unsure
Refrigerator	Yes	No	Unsure
Freezer	Yes	No	Unsure
Autoclave	Yes	No	Unsure
Incubator	Yes	No	Unsure
Blood agar made from non-human blood	Yes	No	Unsure
Commercially prepared powder to prepare selective media	Yes	No	Unsure
Antimicrobial disks	Yes	No	Unsure
Antimicrobial gradient agar diffusion tests (Etests)	Yes	No	Unsure

4. How many people do bench-work in your laboratory? _____

5. Does a trained microbiologist work in your laboratory?
☐ Yes ☐ No ☐Unsure

6. What sources of information do people use regularly in your lab? **(Please check all that apply)**

Textbooks –> please list title and publication date for 2 most frequently used textbooks:

_____
_____
_____

☐ National Committee for Clinical Laboratory Standards (NCCLS/CLSI) tables or guidelines
☐ Standard Operating Procedures specific to your country or laboratory
☐ Laboratory Methods for the Diagnosis of Epidemic Dysentery and Cholera Manual
☐ Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World (picture in letter)
☐ Other manuals → Please list title & publication date:
_____
☐ Internet resources
☐ Something else (please tell us) _____

7. Is your laboratory considered a public health ***reference*** laboratory? (In other words, do you receive referral specimens from other laboratories, for example, to confirm diagnoses for remote laboratories?)

☐ Yes ☐No ☐ Unsure

(IF NO, PLEASE SKIP TO #9)

8. Does your lab serve as the ***primary*** reference lab for antimicrobial susceptibility testing in your country?

☐ Yes ☐ No ☐ Unsure

About Your Laboratory Practice

9. From which of the following places does your lab receive specimens? **(Please check all that apply)**

a) Hospitals b) Clinics c) Private doctors d) Other labs

e) Research f) Other (please tell us where)_____

10. From which place does your lab receive the ***most*** specimens? **(Please check only one)**.

a) Hospitals b) Clinics c) Private doctors d) Other labs
e) Research f) Other (please tell us where)_____

11. Which of the following kinds of pathogens does your lab test for? **(Please check all that apply)**

a) Bacteria that cause pneumonia and meningitis
b) Bacteria that cause sexually-transmitted infections
c) Bacteria that cause enteric diseases of public health concern

d) Anything else? (please tell us)_____

12. Does your lab currently perform antimicrobial susceptibility testing?
☐ Yes ☐ No ☐ Unsure
(***IF NO, PLEASE SKIP TO # 18)***

13. Which of the following types of tests does your lab use for antimicrobial susceptibility testing? (**Please check all that apply)**
- ☐ Disk diffusion (Kirby–Bauer)
- ☐ Disk diffusion (Stokes' method)
- ☐ MIC (Minimum inhibitory concentration testing) using broth microdilution method
- ☐ E-test

14. Do you have written Standard Operating Procedures (SOPs) to describe how to perform antimicrobial susceptibility testing by the method(s) you use? **(Please check only one)**
☐ Yes ☐ No ☐ Unsure
(IF NO, PLEASE SKIP TO #15)

If yes, who wrote or validated them, and when were they written?
_____
_____

15. What source of interpretive criteria do you use when you perform antimicrobial susceptibility testing? (**Please check all that apply)**
☐ NCCLS (National Committee for Clinical Laboratory Standards, USA)
☐ BSAC (British Society for Antimicrobial Chemotherapy)
☐ SFM (Société Française de Microbiologie)
☐ Other (please tell us) _____
☐ No source

16. To whom do you distribute the results of your antimicrobial susceptibility testing, including both routine and surveillance testing as well as quality assessment specimens? **(Please check all that apply)**
☐ Hospitals or clinics ☐ Other health-care providers
☐ Ministry of Health ☐ Organizing lab
☐ Local research team ☐ National surveillance system
☐ International surveillance system
☐ WHO (World Health Organization)
☐ WHO Regional Office for Africa/NHLS EQA (National Health Laboratory Service/External Quality Assessment) Programme
☐ Someone else (please tell us) _____

17. Please indicate **approximately** how many antimicrobial susceptibility tests your lab has performed on isolates of the following organisms in the last **TWELVE MONTHS (ONE YEAR)**. We do not expect an exact number; please just circle your best estimate.

	Number of antimicrobial susceptibility tests performed in your lab on isolates in the last 12 months (one year)					
Haemophilus influenzae	0	1-5	6-25	26-50	51-100	>100
Neisseria meningitidis	0	1-5	6-25	26-50	51-100	>100
Streptococcus pneumoniae	0	1-5	6-25	26-50	51-100	>100
Neisseria gonorrhoeae	0	1-5	6-25	26-50	51-100	>100
Salmonella serotype Typhi	0	1-5	6-25	26-50	51-100	>100
Shigella	0	1-5	6-25	26-50	51-100	>100
Vibrio cholerae	0	1-5	6-25	26-50	51-100	>100

18. Does your laboratory perform ***internal*** quality control for…

a) media preparation? ☐ Yes ☐ No ☐ Unsure
b) bacteriology? ☐ Yes ☐ No ☐ Unsure
c) antimicrobial susceptibility testing? ☐ Yes ☐ No ☐ Unsure

(IF NO TO 18c, PLEASE SKIP TO #19)

If your laboratory DOES practice internal quality control for antimicrobial susceptibility testing, please tell us how:

_____
_____
_____
_____

19. Does your laboratory participate in an External *Q*uality Assessment (EQA) programme for antimicrobial susceptibility testing?

☐ Yes ☐ No ☐ Unsure

(IF NO TO 19, PLEASE SKIP TO #20)

If yes, please tell us which organization provides the EQA programme:

_____
_____

About the Manual

20. Please check this box if you have never seen the manual pictured in the cover letter: ☐

→ IF YOUR LAB NEVER RECEIVED THIS MANUAL, PLEASE STOP NOW AND RETURN THIS SURVEY TO: **Kerrigan McCarthy**

21. How many copies of this manual are there in your laboratory?

_____

22. Is this manual easily accessible to laboratory staff?

☐ Yes ☐ No ☐ Unsure

23. When did you first receive this manual? _____ (month, year)

24. Did you hear about this manual (for example, from the Ministry of Health or a visiting scientist) before or at the same time that you received this manual?
☐ Yes ☐ No ☐ Unsure

(***IF NO, PLEASE SKIP TO # 25)***

If yes, please tell us from whom you heard about the manual. (Please check all that apply)

☐ WHO/PAHO (World Health Organization / Pan-American Health Organization)
☐ WHO Regional Office for Africa/NHLS EQA Programme
☐ CDC (Centers for Disease Control & Prevention)
☐ USAID (United States Agency for International Development)
☐ Ministry of Health ☐ Colleague ☐ Visiting
☐ Scientist
☐ Other (please tell us)_____

25. Did you receive any training about the procedures in this manual at the same time that you received this manual?
☐Yes ☐ No ☐ Unsure

26. During the **first three months** that you had the manual in your laboratory, how often did you and/or other people in your laboratory use the manual? **(Please check only one)**

☐ At least once per day ☐ At least once per week
☐ At least once per month ☐ At least once per 3 months
☐ Not at all during those 3 months

27. During the **last three months** (counting back 3 months from today), how often did you and/or other people in your laboratory use the manual? **(Please check only one)**

☐ At least once per day ☐ At least once per week
☐ At least once per month ☐ At least once per 3 months
☐ Not at all during those 3 months

28. **BEFORE** you received the manual, did your lab ever perform antimicrobial susceptibility tests?
☐ Yes ☐ No ☐ Unsure

29. In your opinion, did the ***number*** of antimicrobial susceptibility tests performed in your lab change after you received the manual? **(Please check only one)**
☐ Yes, we performed **more** tests after we received the manual.
☐ Yes, we performed **fewer** tests after we received the manual.
☐ No, the number of tests we performed **remained the same** as before we had the manual.

30. In your opinion, did the ***accuracy of results*** of antimicrobial susceptibility tests performed in your lab change after you received the manual? **(Please check only one)**
☐ Yes, I believe our test results became **more accurate** after we received the manual.

☐ Yes, I believe our test results became **less accurate** after we received the manual.
☐ No, I believe our test results **did not change** after we received the manual.

31. Did the following laboratory procedures change in any way after you received the manual?

H. influenzae susceptibility testing	Yes	No	Unsure	Not applicable
If yes, please describe how:_____				
N. meningitidis susceptibility testing	Yes	No	Unsure	Not applicable
If yes, please describe how:_____				
S. pneumoniae oxacillin testing to determine penicillin susceptibility	Yes	No	Unsure	Not applicable
If yes, please describe how:_____				
N. gonorrhoeae susceptibility testing	Yes	No	Unsure	Not applicable
If yes, please describe how:_____				
Enteric disease susceptibility testing	Yes	No	Unsure	Not applicable
If yes, please describe how:_____				
Quality control for susceptibility testing	Yes	No	Unsure	Not applicable
If yes, please describe how:_____				
Preparation and/or quality control of media	Yes	No	Unsure	Not applicable
If yes, please describe how:_____				
Safety practices in the lab	Yes	No	Unsure	Not applicable
If yes, please describe how:_____				

32. Please tell us about any other changes in your laboratory since you received the manual.

_____
_____
_____
_____

33. In your opinion, the ***number*** of graphics (such as diagrams, charts, and photographs) in the manual is: **(Please check only 1)**
☐ too many ☐ too few ☐ appropriate

34. Is it easy to understand the English in the manual?
☐ Yes ☐ No ☐ Unsure

35. Would the manual be easier to understand if it were written in a different language?
☐ Yes (**please tell us which language)** _____
☐ No ☐ Unsure

36. Do you or others in your laboratory use any of the appendices in the manual? ☐ Yes ☐ No ☐ Unsure
(***IF NO, PLEASE SKIP TO # 37)***
If yes, please tell us which appendices are particularly useful:
_____
_____
_____

37. What do you like best about the manual?

38. What do you like least about the manual?

39. How does this manual differ from other sources of information used in your laboratory?

40. How could we make a future manual better than this one?

41. Is there anything else about the manual you would like to share with us?

THANK YOU VERY MUCH FOR YOUR PARTICIPATION!
PLEASE RETURN YOUR COMPLETED SURVEY…
BY EMAIL: Heidi Brown, HWBrown@cdc.gov
OR BY MAIL: Ms. Vivian Fensham,
WHO/NHLS QA Programme, National Health Laboratory Service
Watkins-Pitchford Building, P.O.Box 1038
Johannesburg 2000, South Africa
OR BY FAX: +27 11 489 9466 (Attn: Ms. Vivian Fensham)

ANNEX 3

Summary of survey challenges and expected responses for WHO/NICD bacteriology EQA

(July 2002–January 2006)

3.1 Enteric challenges and expected responses

Survey number	2002-1	2002-2	2003-1
Clinical details	A 4-year-old boy presenting with bloody diarrhoea to the local hospital. His 3-year-old cousin has the same symptoms	A 3-year-old boy presenting with bloody diarrhoea and haemolytic uraemic syndrome (HUS)	Rectal swab from a 2-year-old with diarrhoea
Culture and identification	*Escherichia coli,* antimicrobial susceptibility results reported	*Shigella* species	*Vibrio cholerae*
Serotyping	NA	*Shigella dysenteriae* type 1	*Vibrio cholerae* serogroup O1
Antimicrobial selection	NA	Chloramphenicol, ampicillin, trimethoprim–sulphamethoxazole, a quinolone	Trimethoprim–sulphamethoxaole, tetracycline, chloramphenicol furazolidone, ciprofloxacin, ampicillin
Antimicrobial susceptibility	NA	Susceptible to all antimicrobials tested	Susceptible to all 6 of the above antimicrobials

Survey number	2003-2	2003-3	2004-1
Clinical details	Isolate from blood culture from febrile child sent to your laboratory for confirmation of identification	The cook of a large urban restaurant presents with mild diarrhoea. A stool specimen is sent to the laboratory	A 33-year old man presents with dysentery
Culture and identification	*Salmonella* serotype Typhi	*Vibrio cholerae*, with normal flora (+/- *Citrobacter freundii)*	*Shigella* species
Serotyping	Vi antigen and/or O: 9 and H: d antigen positive	Serogroup 01, Ogawa	*Shigella flexneri*
Antimicrobial selection	Ampicillin, ciprofloxacin, chloramphenicol, trimethoprim–sulphamethoxazole, ceftriaxone/ cefotaxime	Any of the following: chloramphenicol, trimethoprim–sulphamethoxazole, furazolidone, tetracycline, nalidixic acid, ciprofloxacin	Ampicillin, chloramphenicol, trimethoprim–sulphamethoxazole, nalidixic acid, ciprofloxacin
Antimicrobial susceptibility	Susceptible to all drugs tested	Susceptible to all except trimethoprim–sulphamethoxazole	Resistant to ampicillin and trimethoprim–sulphamethoxazole; susceptible to chloramphenicol, nalidixic acid, ciprofloxacin

Survey number	2004-2A	2004-3A	2005-1A
Clinical details	Dysentery in a hospitalized 1-year old child	Stool from a 28-year-old female with dysentery (bloody stools) and fever	A 6-month old baby presents with diarrhea and vomiting
Culture and identification	*Salmonella* species	*Escherichia coli,* diarrhoeagenic strain considered	"Normal flora" or "no pathogens isolated" or "non-pathogenic *E coli* isolated"
Serotyping	*Salmonella enterica* subspecies enterica, serovar Isangi or *Salmonella enterica* O: 6,7; H: d, 1,5	O157:H7 or O157	NA
Antimicrobial selection	Amoxicillin/clavulanate, 3rd generation cephalosporin (for ESBL testing), ciprofloxacin/other FQ and any of tetracycline, cotrimoxazole, chloramphenicol	Studies have shown that antimicrobial therapy may worsen the course of the disease +/- AST	NA
Antimicrobial susceptibility	ESBL-producing; susceptible to	Studies have shown that antimicrobial therapy	NA

	fluoroquinolones; and resistant to all other antibiotics that may be used to treat this infection	may worsen the course of the disease +/- AST	
Survey number	**2004-2B**	**2004-3B**	**2005-1B**
Clinical details	Diarrhoea in a clinic nurse	Stool from a 2-year old child who has diarrhoea	A 22-year old woman presents with severe diarrhoea and dehydration
Culture and identification	*Vibrio cholerae*	*Shigella* species	*Vibrio cholerae*
Serotyping	Serogroup O1 or serogroup O1, serotype Ogawa	*Shigella sonnei*	Serogoup O1 serotype Inaba, or Serogroup O1
Antimicrobial selection	Any of tetracycline, furazolidone, trimethoprim–sulphamethoxazole, chloramphenicol, nalidixic acid, ciprofloxacin, tested against *Vibrio cholerae*	Nalidixic acid, trimethoprim–sulphamethoxazole, chloramphenicol, ciprofloxacin, tetracycline, ampicillin, 3rd generation cephalosporin	Any of tetracycline, furazolidone, trimethoprim–sulphamethoxazole, chloramphenicol, nalidixic acid, ciprofloxacin
Antimicrobial susceptibility	Susceptible to tetracycline, chloramphenicol, nalidixic acid, ciprofloxacin; resistant to trimethoprim–sulphamethoxazole	Susceptible to tetracycline, chloramphenicol, nalidixic acid, ciprofloxacin, ampicillin; resistant to trimethoprim–sulphamethoxazole	Susceptible to all of the above antibiotics

Survey number	**2005-2A**	**2005-3A**	**2006-1A**
Clinical details	Stool from acutely febrile five-year old with mild diarrhoea	Bloody diarrhoea in a 30-year old male	A 25 year-old man with diarrhoea
Culture and identification	*Salmonella* species	*Shigella* species	*Aeromonas hydrophila*
Serotyping	*Salmonella* Paratyphi C (Vi +ve/ Vi -ve)	*Shigella dysenteriae* type 2	Not applicable
Antimicrobial selection	Ampicillin, co-amoxyclav, 3rd generation cephalosporin, , ciprofloxacin / nalidixic acid, cotrimoxazole, chloramphenicol and tetracycline	Nalidixic acid, trimethoprim-sulphamethoxazole, ampicillin, ciprofloxacin, chloramphenicol	Ampicillin, co-amoxyclav, 3rd generation cephalosporin, , ciprofloxacin / nalidixic acid, cotrimoxazole, chloramphenicol and tetracycline
Antimicrobial	Susceptible to all	Susceptible to all tested	Resistant to ampicillin,

susceptibility	tested +/- ESBL negative		susceptible to other antibiotics
Survey No	**2005-2B**	**2005-3B**	**2006-1B**
Clinical details	Three-year old patient who developed diarrhoea during an outbreak of gastroenteritis at a day-care center.	A patient with diarrhoea and severe dehydration in a refugee camp	Stool specimen from a health-care worker during screening for outbreak of nosocomial infections in an ICU.
Culture and ID	*Shigella* species	*Vibrio cholerae*	*Salmonella* species
Serotyping	*Shigella boydii*	*Vibrio cholerae* serogroup O1 serotype Ogawa	*Salmonella* enterica subspecies enterica serotype Muenchen
Antimicrobial selection	Any of ampicillin, cotrimoxazole, chloramphenicol, nalidixic acid and ciprofloxacin	Nalidixic acid, trimethoprim-sulphamethoxazole, furazolidone, ciprofloxacin, tetracycline	Ampicillin, co-amoxyclav, 3rd generation cephalosporin, , ciprofloxacin / nalidixic acid, cotrimoxazole, chloramphenicol and tetracycline
Antimicrobial susceptibility	Susceptible to all tested	Susceptible to all tested	Susceptible to all tested

NA, not applicable; ESBL, extended-spectrum beta-lactamase; AST, antimicrobial susceptibility testing

Comment on enteric challenges:

The organisms in the enteric surveys were selected on the basis of their role as major public health pathogens. The first organism, a commensal *Escherichia coli,* was chosen to ascertain whether the courier and transport systems were able to handle live pathogens, and to test laboratories' ability to identify a frequently isolated, non-pathogenic bacterium. The history and presentation of the challenges has increased in complexity. All surveys were sent as simulated stool (prepared from sterilized lentils, inoculated with the pathogen).

Simulated stool from surveys 2002-2, 2003-1 and 2003-2 were inoculated with only one organism, while survey 2003-3 was inoculated with two organisms, only one of which was pathogenic (*Vibrio cholerae*). The number of enteric challenges was increased to two per survey from the third year of the programme. Internal QC indicated that viability of *Vibrio cholerae* was limited and consequently the organism was included on semi-solid agar in the shipment in Survey 2005-1. Enterohaemorrhagic *E. coli* O157:H7 was included on account of the organism being an emerging pathogen on the African continent. A specimen containing only normal flora was included as a "distracter" in survey 2005-1. In year 4, *Salmonella* species, and *Shigella* species were both included twice, *Vibrio cholerae* once, and *Aeromonas* species once.

3.2 Meningitis challenges and expected responses

Survey number	2002-1	2002-2	2003-1
Clinical details	**B.** A 22-year old man presenting with meningitis. On examination he is wasted and has generalized lymphadenopathy **C.** A 2-year old girl presenting to the hospital with neck stiffness. Her aunt brought her to hospital and she does not know what vaccinations the child has received	2-year old female patient seen at outlying clinic. No further clinical information submitted. The primary health care nurse at the clinic prepared the CSF smear and inoculated the TI media with CSF specimen	4-week old boy presents with vomiting and lethargy. The attending doctor examined the child at the weekend, she inoculated the TI medium, made a smear with the child's CSF and sent it to the laboratory for processing
Microscopy	**B.** Gram-positive diplococci, presumptive for *Streptococcus pneumoniae* **C.** Gram-negative diplococci, presumptive for *Neisseria meningitidis*	Neutrophils and Gram-negative coccobacilli suggestive of *Haemophilus influenzae*	Gram-positive cocci in chains, suggestive of S*treptococcus* species
Culture and identification	NA	*Haemophilus influenzae*	Group B streptococcus, (*Streptococcus agalactiae*)
Serotyping	NA	*Haemophilus influenzae* type b	NA
Antimicrobial selection	NA	Ampicillin and/or β-lactamase test, chloramphenicol, ceftriaxone/cefotaxime	Susceptibility testing not routinely indicated; penicillin is the drug of choice
Antimicrobial susceptibility	NA	β-lactamase positive, resistant to ampicillin, chloramphenicol; susceptible to ceftriaxone /cefotaxime	Susceptible to penicillin and ampicillin

Survey number	2003-2	2003-3	2004-1
Clinical details	A 20-year old woman presented to a clinic with a severe headache. She was transferred to the regional hospital, where a lumbar puncture is performed. A slide and a TI medium bottle are forwarded on to your laboratory	A 34-year old HIV-seropositive female presented with a cough and confusion. A cell count performed on her CSF demonstrates 1250 neutrophils/mm^3 and 108 lymphocytes/mm^3. An unstained slide of her CSF and TI bottle inoculated at the bedside were submitted to your laboratory for further testing	Specimen form, sent with slide and TI bottle, states: " Unknown age, male, patient unable to give history". Referral laboratory did not perform microscopy or biochemistry
Microscopy	Gram-negative diplococci	Gram-positive diplococci	Gram-positive diplococci
Culture and identification	*Neisseria meningitidis*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*
Serotyping	W135	23F	NA
Antimicrobial selection	Any of penicillin, amoxicillin, rifampicin, trimethoprim-sulphamethoxazole, chloramphenicol, and a 3rd generation cephalosporin and/or β-lactamase test	Penicillin, cefotaxime/ceftriaxone, trimethoprim–sulphamethoxazole, chloramphenicol, rifampicin, tetracycline	Penicillin, ceftriaxone/cefotaxime MIC/Etest
Antimicrobial susceptibility	E-test result for above antibiotics, and/or β-lactamase negative	Penicillin-resistant by MIC; Ceftriaxone-intermediate by MIC; resistant to trimethoprim–sulphamethoxazole, chloramphenicol, tetracycline; susceptible to rifampicin	Penicillin-resistant; ceftriaxone-intermediate

Survey number	2004-2C and D	2004-3C	2005-1C
Clinical details	The isolate had been identified as a *Neisseria meningitidis* in an outlying laboratory. It was sent to your laboratory for further serogrouping	CSF from a 3-year old boy with meningitis	A thirty-year-old male who has AIDS presents with headache, vomiting and fever
Microscopy	NA	Gram-negative coccobacilli or pleomorphic rods	Yeast cells
Culture and identification	NA	*Haemophilus influenzae*	*Cryptococcus neoformans* or *Cryptococcus* species
Serotyping	**2C** - *Neisseria meningitidis* serogroup A **2D** - *Neisseria meningitidis* serogroup W135	Autoagglutination (non-typeable by PCR)	NA
Antimicrobial selection		Ampicillin, 3rd generation cephalosporin, chloramphenicol, -/+ β-lactamase test	NA
Antimicrobial susceptibility		Susceptible to ampicillin, ceftriaxone, chloramphenicol; β-lactamase negative	NA
Survey number	**2004-2F**	**2004-3D**	**2005-1D**
Clinical details	The isolate had been identified as a *Streptococcus pneumoniae* in an outlying laboratory. It was sent to your laboratory for antimicrobial susceptibility testing	CSF from an adult with meningitis	An eighteen-year-old soldier presents with headache, neck stiffness and a petechial rash
Microscopy	NA	Gram-positive coccobacilli / short bacillus / bacilli	Gram-negative diplococci
Culture and identification	NA	*Listeria monocytogenes*	*Neisseria meningitidis*
Serotyping	NA	NA	Serogroup B
Antimicrobial method/selection	Acceptable, according to NCCLS/SFM/BSAC procedure	Cotrimoxazole, ampicillin, gentamicin	Penicillin/ampicillin/oxacillin, ciprofloxacin/ofloxacin, sulphonamide, rifampicin, chloramphenicol, +/- 3rd generation cephalosporins, +/- β-lactamase

Antimicrobial susceptibility	Oxacillin zone diameter < 20mm (NCCLS/BSAC) or < 26mm (SFM); penicillin MIC/E-test done or referred; result = 2 µg/ml (range1–4 µg/ml); interpretation = high level resistance to penicillin	Susceptible to ampicillin, gentamicin, cotrimoxazole	Guidelines stated (CLSI(NCCLS)/SFM/BSAC) and respective methodology followed correctly. Penicillin MIC = 0.047 µg/ml (susceptible), sulphonamide MIC = 4 µg/ml (resistant), rifampicin MIC = 0.094µg/ml (susceptible), ciprofloxacin, MIC = 0.002µg/ml (susceptible), oxacillin disc susceptible (SFM only)

Survey number	2005-2C	2005-3C	2006-1C
Clinical details	18-month old infant who presented with pyrexia and a generalized seizure. CSF was clear with no cells and normal glucose and protein	Fever and neck stiffness in an 8-month old infant.	A 9-month-old child is admitted with lethargy and vomiting.
Microscopy	No organisms seen	Gram-negative cocco-bacilli	Gram-positive diplococci
Culture and identification	Viridans group *Streptococcus* with comment to clinician	*Haemophilus influenzae*	*Streptococcus pneumoniae*
Serotyping	NA	*Haemophilus influenzae* serotype c	NA
Antimicrobial method/selection	Not evaluated	Ampicillin,co-amoxiclav, ceftriaxone, chloramphenicol	Penicillin (MIC), ceftriaxone/cefotaxime (MIC), oxacillin (disc), chloramphenicol, vancomycin
Antimicrobial susceptibility	Not evaluated	Guidelines stated, correct methodology, correct results, susceptible to all tested	Guidelines stated, correct methodology, resistant to penicillin, cefotaxime, susceptible to chloramphenicol, vancomycin

Survey number	2005-2D	2005-3D	2006-1D
Clinical details	A 2-year-old female child with acute onset of fever, headache and neck stiffness.	An unconscious, pyrexial 9-year-old child.	A 18-month-old boy presents with loss of consciousness and pyrexia.
Microscopy	Gram-positive diplococci / gram-positive cocci +/- polys	Gram-negative diplococci/ cocci	Gram-negative coccobacilli
Culture and identification	*Streptococcus pneumoniae*	*Neisseria meningitidis*	*Haemophilus influenzae*
Serotyping	NA	Serogroup C	*Haemophilus influenzae* serotype f
Antimicrobial method/selection	Penicillin and ceftriaxone/cefotaxime MIC/Etest; Chloramphenicol, vancomycin and rifampicin discs	Penicillin/ampicillin, +/- ceftriaxone/cefotaxime, chloramphenicol, rifampicin, ciprofloxacin	Ampicillin, ceftriaxone, chloramphenicol, trimethoprim-sulphamethoxazole, ofloxacin, meropenem
Antimicrobial susceptibility	S to all tested, oxacillin susceptible or Pen MIC within susceptible range	Guidelines stated, correct methodology, S to all tested	Guidelines stated, correct methodology, β-lactamase positive, ampicillin resistant, susceptible to other antibiotics tested

CSF, cerebrospinal fluid; TI, trans-isolate medium; NA, not applicable; NCCLS, national committee for clinical laboratory standards; BSAC, British Society for Antimicrobial Chemotherapy; SFM, Société Française de Microbiologie; CLSI, Clinical Laboratory Standards Institute

Comment on meningitis challenges:

The organisms in the meningitis surveys were selected on the basis of their role as major public health pathogens. The first challenge consisted of two simulated CSF slides. Subsequently, each challenge consisted of a simulated slide of CSF and a TI inoculated with the pathogen. TI is a biphasic medium that can be inoculated with CSF at the bedside. It is capable of sustaining the growth of small numbers of the common pathogens that cause meningitis. TI is useful where there is a delay in transporting CSF to the laboratory and is used in the field by several participating laboratories. The TI performed well, with the exception of survey 2003-3, when laboratory quality control procedures indicated that growth of the *Streptococcus pneumoniae* tailed off 18 days after inoculation. A substantial proportion (17/38, 45%) of laboratories failed to obtain growth. The evaluation of laboratory responses was adjusted accordingly.

Problems with susceptibility testing of pneumococci were identified in Survey 2003-3, and the subsequent survey included two identified strains of pneumococci on which laboratories were requested to perform susceptibility testing only. A non-typeable *Haemophilus influenzae* that auto-agglutinated in saline was included in order to emphasize the importance of performing controls. *Cryptococcus neoformans* and *Listeria monocytogenes* were included in Survey 2004-3D and 2005-1C to challenge

laboratories' abilities to identify uncommon pathogens associated with HIV co-infection.

In the fourth year the following organisms were included: *Neisseria meningitidis*, *Streptococcus pneumoniae* (twice), *Haemophilus influenzae* (twice), and a viridans S*treptococcus*, included to simulate a contaminated CSF and test laboratories' response to this scenario.

3.3 Plague challenges and expected responses

Survey number	2002-1	2002-2	2003-1
Clinical details	A traveler, recently returned from India, developed a fever. On examination inguinal lymphadenopathy was detected	Serum samples 1 and 2 collected during plague field surveillance activities	Lymph node aspirate from a rural Zambian resident who presented with fever and painful inguinal lymphadenopathy. Bubonic plague was diagnosed clinically and antibiotics were given before the specimen was obtained for laboratory confirmation
Microscopy	Bipolar staining, presumptive for *Yersinia pestis*	NA	NA
Serology	NA	Serum sample 1: Positive Serum sample 2: Negative	Positive for F1 antigen

Survey number	2003-2	2003-3	2004-1
Clinical details	Lymph node aspirate from a rural Zambian resident who presented with fever and painful inguinal lymphadenopathy. Bubonic plague was diagnosed clinically and antibiotics were given before this specimen was obtained for laboratory confirmation	Isolated from a septicaemic patient in a plague endemic area	Isolated from a septicaemia patient in a plague endemic area
Microscopy	NA	NA	NA
Culture and identification	NA	*Yersinia enterocolitica*	*Klebsiella pneumoniae*
Serology	Positive for F1 antigen	NA	NA

Survey number	2004-2G & 2004-2H	2004-3E	2005-1E
Clinical details	Lymph node aspirates from two family members who are residents of a rural town. They presented with fever and painful inguinal lymphadenopathy. Bubonic plague was suspected clinically and antibiotics were given before these specimens were obtained for laboratory confirmation.	A traveler, recently returned from India, developed a fever; on examination inguinal lymphadenopathy was detected	This traveler presented with a clinical picture of septicemia and this organism was recovered from a blood culture
Microscopy	NA	Gram-negative bacilli / coccobacilli/with or without bipolar staining presumptive for *Yersinia pestis*	NA
Culture and identification	NA	NA	*Yersinia enterocolitica*
Serology	2G - Positive for F1 antigen 2H - Negative for F1 antigen	NA	NA

Survey number	2005-1F
Clinical details	Organism isolated from a rodent during plague surveillance
Microscopy	NA
Culture and identification	*Pasteurella multocida*
Serology	NA

Survey number	2005-2E	2005-3E	2006-1E
Clinical details	NA	Fever and swelling in the groin in a male adult	Backpacker returning from Uganda with septicaemia
Microscopy	NA	NA	Bipolar-staining Gram-negative bacilli, presumptive for *Yersinia pestis*
Culture and identification	*Pasteurella multocida*	NA	NA
Serology	NA	F1 antigen negative	NA

Survey No	2005-2F	2005-3F	2006-1F
Clinical details	NA	A traveler, recently returned from India, developed a fever.	Namibian resident with unexplained fever
Microscopy	NA	NA	Bipolar-staining Gram-negative bacilli, presumptive for *Yersinia pestis*
Culture and identification	*Klebsiella pneumoniae*	NA	NA
Serology	NA	F1 antigen negative	NA

NA: Not applicable

Comment on plague challenges:

Challenges were selected on basis of diagnostic tests thought to be available in regional laboratories of the WHO African Region. According to international air transport regulations, it is difficult to transport *Yersinia pestis*; thus this organism cannot be included in the surveys. Simple staining, culture and identification procedures were tested using prepared slides and a culture of *Yersinia enterocolitica*. F1 antigen dipstick tests are currently manufactured in Madagascar by the Pasteur Institute and were distributed to participating laboratories via the EQA courier service. An identical simulated specimen (bubo aspirate) was included in subsequent surveys to evaluate laboratory performance with this dipstick tests over time.

Survey material included slides prepared from *Yersinia pestis* which laboratories were required to stain and comment on the presence of bipolar staining bacilli. Organisms having similar biochemical features to *Yersinia pestis* such as *Pasteurella multocida, Yersinia enterocolitica* and *Klebsiella pneumoniae* were included.

Included in the fourth year were slides for staining (twice), F1 detection by rapid dipstick tests (twice) and organisms (*Pasteurella multocida*, *Klebsiella pneumoniae*) for exclusion of plague agent.

3.4 Malaria microscopy challenges and expected responses

Eight challenges were included in the malaria microscopy discipline, as either thin Giemsa-stained blood films, or thin and thick Giemsa-stained films. Laboratories were required to identify the parasite species present.

Challenge number	Survey No	
	2005-3	2006-1
M1	Not evaluated	*Plasmodium falciparum*
M2	No parasite(s) seen	*Plasmodium falciparum*
M3	*Plasmodium falciparum*	*Plasmodium ovale*
M4	*Plasmodium falciparum*	*Plasmodium falciparum*
M5	*Plasmodium falciparum*	*Plasmodium falciparum*
M6	No parasite(s) seen	No parasite(s) seen
M7	Not included	*Trypanosoma brucei* species
M8	Not included	*Plasmodium falciparum*

Comment on malaria challenges:

The number of challenges per survey was later increased to 10. Each challenge shall consist of a thick and thin Giemsa-stained blood film. The accuracy and consistency of parasite density quantitation shall also be assessed.

3.5 Acid Fast Bacilli microscopy challenges and expected responses

Four AFB microscopy slides are submitted already stained, while four are unstained; participating laboratories are required to stain the unstained slides using a stain of their choice (Ziehl-Neelsen or Auramine-O) for the detection of acid-fast bacilli. Only 7 slides were submitted in the first survey, and 8 in the second. Laboratories are required to report on the presence of acid-fast bacilli using the International Union of Tuberculosis and Lung Disease (IUATLD) grading system.

Survey No	2005-3 (IUATLD grading)	2006-1 (IUATLD grading)
T1	Negative	Negative
T2	2+	2+
T3	2+	1+
T4	Negative	2+
T5	2+	2+
T6	Negative	1+
T7	Negative	2+
T8	Not included	Negative

ANNEX 4: Standard Operating Procedures

Annex 4A: Standard operating procedures for bacteriology EQA

Annex 4B: Standard operating procedures for AFB microscopy EQA

Annex 4C: Standard operating procedures for malaria microscopy EQA

Annex 4A
Standard operating procedures for bacteriology EQA

Procedure WHO 0001	**ISOLATE SUBMITTED FOR INCLUSION IN A PANEL IN THE WHO/NICD EQA PROGRAMME**

Prepared by	**Date adopted**	**Signature**
Vivian Fensham/ Anne von Gottberg		

Revision date	**Revision signature**	**Review date**	**Review signature**

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Date isolate subcultured:	**Intended survey number:**
Unit submitting isolate:	**Tested by:**

Documented identification of isolate: _____
Source of isolate: _____
Laboratory number/s: _____
Date received: _____
Method of storage: _____

Identification (include primary reactions from catalase, oxidase, etc. to final differentiating tests):
Gram staining:_____
Microscopic morphology:_____

Test	**Result**	**Test**	**Result**

API Code (attach strip): _____

Serotyping:

Reagent used (batch):	
Reaction:	**Comment on quality of reaction:**

Antibiotic Susceptibility Testing (disc diffusion & MICs; please document whether E-test or agar dilution or other method used):

Isolate name	Zone size (mm)	S/I/R (disc)	MIC (mg/l)	S/I/R (MIC)

Control strain results within range and recorded: Y ☐ /N ☐

β-lactamase tested: Y ☐ /N ☐ **β-lactamase positive: Y ☐ /N ☐**

Other tests or comments:

Date completed: **Signature:**

Procedure WHO 0002	**INTER-LABORATORY QUALITY CONTROL OF SMEARS PREPARED FOR THE WHO/NICD EQA PROGRAMME**	
Prepared by	**Date adopted**	**Signature**
Vivian Fensham/ Anne von Gottberg		

Revision date	**Revision signature**	**Review date**	**Review signature**

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Quality control for slides for survey no: _____

Slides made on: _____

Submitted to: _____

Date sent: _____

Please note (for CSF smears) smear is heat-fixed but unstained.
Please note (for AFB smear microscopy) smears are heat-fixed; one slide has already been stained and one is to be stained by the laboratory following their own methods.

History given to participants (only applicable if CSF smears):

Sample	**Source**	**Clinical details**	**Instructions**

Please comment on suitability of slide:

Quality of smear:
Characteristics of neutrophils:
Characteristics of epithelial cells:
Characteristics of bacteria:
Any other comments:

Name:_____

Date:_____

Procedure WHO 0003	**SIMULATED ENTERIC SPECIMEN IN CARY–BLAIR MEDIUM**		
Prepared by	**Date adopted**	**Signature**	
Vivian Fensham/ Anne von Gottberg			

Revision date	**Revision signature**	**Review date**	**Review signature**

Distributed to	**Number of copies**	**Distributed to**	**Number of copies**
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Purpose:

To prepare a simulated stool specimen by mixing lentils with the pathogen (e.g. *Shigella dysenteriae* type 2) in Cary–Blair transport medium (with or without a cotton-tipped swab).

Selection of pathogen:

Isolates can be selected from stored clinical isolates or reference organisms – specific isolates that demonstrate aspects of identification, serotyping/grouping, or susceptibility testing are chosen:

1. Technical Implementation Group (TIG) decision
2. Isolate (with laboratory or control strain number, date received in specialist laboratory) submitted from specialist laboratory with working card (see separate SOP WHO0001) confirming identification, serotyping/grouping, susceptibility testing as appropriate (β-lactamase testing, disc diffusion, MICs)
3. Independent quality control performed in EQA laboratory as required – discrepant results are queried at the level of the specialist laboratory (consider independent clinical laboratory – Infection Control Laboratory/Microbiology Laboratory at Johannesburg General Hospital and/or Microbiology Laboratory at Chris Hani Baragwanath Hospital).

Lentil preparation:

1. **Day 1:** Boil lentils, and then blend into a pulp (this can be done at home).
2. **Day 2:** Weigh out 42g of cooked lentils into each of 5 beakers. If the mixture is dry, add sterile water before autoclaving. Cover beakers with aluminum foil and autoclave for 15 minutes. Cool the mixture and stored in the refrigerator.
3. Each beaker yields sufficient material for about 35 Cary–Blair medium bottles.
4. Have some sterile water or broth on hand, after autoclaving the mixture can be too dry and the addition of water or broth will make the lentil mixture creamier.

Organism suspension preparation:

1. **Day 1**: Inoculate 2–3 blood agar plates with a pure culture of the organism and incubate overnight at 37 ºC under aerobic condition to obtain heavy growth.

2. **Day 2:** Take a heavy sweep of the organism from the blood agar with a sterile swab and emulsify in 6 ml of Mueller-Hinton broth, in a sterile plastic centrifuge tube.
3. Vortex.
4. Confirm an optical density of 0.8 at 570 wavelength (McFarland 4), equivalent to 12 $\times 10^8$ cfu/ml, using a spectrophotometer, and Mueller-Hinton as a blank control
5. Adjust to obtain above optical density reading by adding Mueller-Hinton or adding more of the organism emulsion.
6. This is the working bacterial suspension.

Method for inoculating the Cary–Blair medium:

1. Dispense 2 ml of the bacterial suspension into each beaker containing 42 g of lentils.
2. Mix carefully using a sterile applicator stick or spatula.
3. Inoculate generous pea-sized portions of the lentil and organism mixture into each Cary–Blair vial using either a sterile swab or sterile disposable pipette.
4. Insert cotton-tipped swab, if required, and break off shaft to close bottle.
5. Seal by closing the screw cap top.
6. Store at room temperature until shipping.

Quality control:

1. A sterility check must be carried out on all media and reagents used in the preparation of the organism and lentils.
2. The lentils must be checked for sterility after autoclaving and prior to the addition of the organism.
3. The lentil and organism mixture is plated onto non-selective media at the start and at the end of the inoculation of the Cary–Blair media. This is to confirm growth as well as check for contamination of the samples.
4. All inoculated bottles are stored at room temperature until shipping.
5. Timing of quality control (if 90 specimens are prepared: 68 samples are sent, 22 bottles are kept for quality control):
    - 5 bottles are sub-cultured at weekly intervals;
    - all bottles are numbered, identifying at which stage they were sub-cultured;
    - at closure of a shipment (when the EQA TIG decides that no more responses are outstanding or expected), all bottles are sub-cultured;
    - bottles are kept in the EQA laboratory until a review of responses and quality control results are reconciled.
6. Failure of QC:
    - need 100% compliance;
    - any non-viability of pathogen with initial sub-culture prior to inoculation in Cary-Blair constitutes failure (for mixed cultures – need to evaluate predominance of pathogen);
    - hold shipment, repeat test;
    - if repeat test consistent with the first non-compliance, stop shipment, evaluate necessary corrective action and start from the beginning of the procedure;
    - subsequent bottles are tested for viability over time; non-viability will determine the inability to evaluate laboratories with no growth;
    - sometimes it may be necessary to include a semisolid or lyophilized pure culture of the pathogen in suitable transport media, if there is any doubt of the pathogen's survival over prolonged time.

WORKING CARD

QUALITY CONTROL FOR SIMULATED STOOL SPECIMENS

Survey no: _____
Organism used: _____
Optical density reading: _____
Preparation date: _____

1. Sterility check (on blood agar)

Sample	24 h	48 h
a. Mueller-Hinton Broth		
b. Lentils after autoclaving		
c. Lentils with organism prior to inoculation		
d. Lentils with organism after inoculation		

2. Viability of the isolate

Sample No	Date of subculture	Date read	All media is incubated for 24–48 h at 37 ºC					
			Blood agar	Mac	XLD	DCA	SS	TCBS
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

Procedure WHO 0004	**MENINGEAL PATHOGEN IN TRANS-ISOLATE (TI) TRANSPORT MEDIUM**	
Prepared by	**Date adopted**	**Signature**
Vivian Fensham/ Anne von Gottberg		

Revision date	**Revision signature**	**Review date**	**Review signature**

Distributed to	**Number of copies**	**Distributed to**	**Number of copies**
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Purpose:

To simulate a cerebrospinal fluid specimen with a specific pathogen.

Selection of pathogen:

Isolates can be selected from stored clinical isolates or control organisms – specific isolates that demonstrate aspects of identification, serotyping/grouping, or susceptibility testing are chosen:

1. Technical Implementation Group (TIG) decision.
2. Isolate (with laboratory or control strain number, date received in specialist laboratory) submitted from specialist laboratory with working card (see separate SOP WHO0001) confirming identification, serotyping/grouping, susceptibility testing as appropriate (β-lactamase testing, disc diffusion, MICs).
3. Independent quality control performed in EQA laboratory as required – discrepant results are queried at the level of the specialist laboratory (consider independent clinical laboratory – Infection Control Laboratory/Microbiology Laboratory at Johannesburg General Hospital and/or Microbiology Laboratory at Chris Hani Baragwanath Hospital).

Isolate preparation:

1. Subculture the isolate onto 3–4 chocolate agar or blood agar plates (non-selective medium) and incubate overnight at 37 ºC in CO_2 5% or aerobically (optimal incubation dependent on pathogen).
2. Observe for pure growth after incubation.
3. Perform sterility quality control of Mueller-Hinton stock broth or other suitable broth and TI medium (2 bottles uninoculated).
4. Inoculate a sweep of the organism (using a sterile cotton wool swab) into a bottle with 100 ml, or suitable volume, of broth. This volume is dependent on the number of samples that need to be prepared.

5. Read the turbidity of the inoculated broth on a spectrophotometer at 570 nm and record the result on the working card; ideally an optical density of 0.8 is suitable for most organisms.
6. Of this suspension, 0.5–1 ml is then inoculated into each TI medium bottle.

Method:

1. Work in a class II biosafety cabinet;
2. remove the aluminium flip top, clean with an alcohol swab and flame if necessary;
3. inoculate each TI medium with 0.5–1 ml of the bacterial suspension using a sterile syringe and needle;
4. incubate in ambient air at 37 ºC for 24 h;
5. the samples need to be checked for contamination after 24 h incubation. To do this 5 bottles are removed at random from the batch, a 0.1 ml sample of TI broth is removed aseptically and planted onto blood agar and incubated for 24 h; these bottles will be labelled quality control (QC) samples 1–5 and kept with further QC samples;
6. include 2 bottles of TI medium that remain uninoculated, but otherwise go through the same procedure as the other bottles (incubation, venting, removal of needles); this is a quality control of the TI medium;
7. the rest of the batch is vented with sterile Airway® needles (distributed by Organon Teknika Corporation); the tops of the bottles are first cleaned with an alcohol swab before inserting the venting needle;
8. leave on the bench until dispatch (ideally this should be no longer than 5 days);
9. remove the venting needle before dispatch;
10. prior to dispatch (the day before) another 5 QC samples are removed at random (QC samples 6–10) and treated as the first five (see 5. above); this is to check for viability and possible contamination during venting;
11. confirm the biochemical reactions and/or serotyping of the organism at this stage.

Quality control:

(Usually prepare samples for 77 laboratories; prepare an additional five for possible reshipments or accidents, thus total of at least 80 per specimen are prepared for shipping and an additional 30 for QC).

1. Timing of QC (30 bottles in total):
   - 5 bottles after 24 hours incubation at 37 ºC;
   - 5 bottles one day before shipment (after venting);
   - all bottles to be kept at room temperature, unvented for the duration of the shipment, sample 5 bottles twice a week for viability (Mon and Thurs);
   - number all bottles, identifying at which stage they were sub-cultured;
   - at closure of a shipment (when the EQA TIG decide that no more responses are outstanding or reasonably expected), all bottles are sub-cultured;
   - bottles are kept in the EQA laboratory until a review of responses and QC results are reconciled.
2. All bottles determined for QC have their venting needles removed at the same time as the shipped bottles.
3. Failure of QC:
   - need 100% compliance;

- any contamination or non-viability of pathogen in bottles 1–10 constitutes failure; hold shipment, repeat test;
- if repeat test consistent with the first non-compliance, stop shipment, evaluate necessary corrective action and start from the beginning of the procedure;
- subsequent bottles are tested for viability over time: non-viability will determine the inability to evaluate laboratories with no growth;
- during the QC period, if any sample shows no growth, all 30 QC samples must be checked for viability immediately.

Method for routine sub-culture from TI medium bottles:

1. clean the top of the bottle with an alcohol swab and flame if necessary;
2. using a sterile needle and syringe, aspirate 0.1 ml of the liquid;
3. place the aspirated material directly onto the appropriate media;
4. incubate at 37 ºC for 24 h under CO_2 5% or aerobically (optimal incubation dependent on pathogen).

Method for sub-culture from TI medium bottles if there has been no growth in one or more samples:

1. clean the top of the bottle with an alcohol swab and flame if necessary;
2. using sterile needle and syringe, aspirate 1 ml of the liquid into a sterile plastic tube;
3. centrifuge the sample and pour off the supernatant;
4. inoculate a blood or chocolate agar plate (90 mm) with the deposit of the centrifuged aspirate, and plate out for single colonies;
5. incubate at 37 ºC for 24 h under CO_2 5% or aerobically (optimal incubation dependent on pathogen);
6. if after 1 ml has been removed, spun and cultured, the sample still shows no growth, then remove all the supernatant from the respective bottle, spin down and plate the deposit;
7. repeat the above method on any future specimens that show no growth until such time as all specimens are non-viable.

WORKING CARD

QUALITY CONTROL OF INOCULATED TI MEDIUM

Survey no: _____
Organism used:_____
Optical density reading:_____
Date of preparation: _____

Minimal sterility of all media and reagents tested; only use incubation at 37 ºC for 48 h aerobically (more extensive sterility testing is performed before release from DMP (Diagnostic Media Products; NHLS, South Africa); TI media also have sterility testing performed at DMP, but no growth performance testing is done. Growth performance of the TI medium is at present done at NICD Respiratory and Meningeal Pathogens Reference Unit and the NICD EQA unit, as appropriate).

Quality control of uninoculated media:

Media/reagents	Date of subculture	Date read	24 h incubation	48 h incubation
Mueller-Hinton broth, serum broth or BHI				
TI medium (2 bottles) uninoculated				

Quality control of inoculated media:

Media/reagents	Date of subculture	Date read	24 h incubation	48 h incubation
Mueller-Hinton broth, serum broth or BHI inoculated with organism				

Quality control of samples sent to participating laboratories:

All samples to be sub-cultured on appropriate media e.g. 5 % blood or chocolate agar

Sample no	Date of subculture	Date read	Growth	Closing date	Growth all samples
1 (after 24 h at 37 ºC)					
2 (after 24 h at 37 ºC)					
3 (after 24 h at 37 ºC)					
4 (after 24 h at 37 ºC)					
5 (after 24 h at 37 ºC)					
6 (1 day before shipment)					
7 (1 day before shipment)					
8 (1 day before shipment)					
9 (1 day before shipment)					
10 (1 day before shipment)					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					

Procedure WHO 0005	**LYOPHILIZED SPECIMENS**

Prepared by	**Date adopted**	**Signature**
Vivian Fensham/ Anne von Gottberg		

Revision date	**Revision signature**	**Review date**	**Review signature**

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Preparation of isolate:

Day 1:

1. Isolate submitted from specialized unit with a working card **or** EQA control isolate sub-cultured from −70 °C with same working card completed by EQA staff.
2. Obtain 10% skim milk with 5% inositol from Diagnostic Media Products (DMP).
3. Quality control for sterility of skim milk used (subculture an aliquot of skim milk onto non-selective media and incubate at room temperature at 37 °C for 48 hours).

Day 2:

1. Subculture the organism onto c. 20 agar plates (number dependent on organism and quantity required).
2. Incubate for 24 hours at conditions optimal for the growth of the organism.

Day 3:

1. Observe for any growth on sterility QC subcultures from Day 1 (after 48 hours incubation).
2. Observe Day 2 plates of the isolate to be lyophilized for pure growth.
3. Working in a class 2 biosafety cabinet, wash the organism off the plates into the skim milk using a sterile swab for each plate.
4. Flame the bottle neck of the skim milk bottle after each washing to ensure sterility.
5. Quality control the stock suspension of bacteria and skim milk by subculturing an aliquot of suspensions (skim milk with bacteria after washing off, before dispensing and after dispensing) onto non-selective media and incubating under

conditions optimal for the growth of the isolate, as well as at room temperature and at 37 °C.

6. Dispense 1.0 ml volumes of the suspension into sterile 6 ml glass vials and cap with sterile rubber stoppers.
7. The vials are ready for freeze-drying.
8. Place vials in −70 °C freezer for one hour, while the freeze-drier is set up.
9. Vials are left in the freeze-drier overnight: ensure that the temperature and vacuum gauges are at correct settings.

Day 4:

1. Seal all vials under vacuum.
2. Cap with aluminium cap.
3. Re-suspend the appropriate number of vials for QC with 1.0 ml of appropriate broth.
4. Incubate for 30 minutes.
5. Subculture the sample after it has been lyophilized to confirm growth and contamination; number of vials to be sampled depends on number to be sent.

Quality control:

1. A sterility check must be carried out on all media and reagents used in the preparation of the organism.
2. The skim milk must be checked for sterility after autoclaving and prior to the addition of the organism.
3. Timing of QC (approximately 100 specimens are prepared; 80 samples need to be sent, 5 additional need to be made for replacements, the rest of the vials are kept for QC).
   - 3 vials are sub-cultured immediately after the batch has been prepared, check for predominant growth on selective and non-selective media.
   - The rest of the QC vials are kept at room temperature and are sub-cultured at weekly intervals.
   - All vials are numbered, identifying at which stage they were sub-cultured.
   - At closure of a shipment (when the EQA panel decide that no more responses are outstanding or reasonably expected); the number of vials remaining are evaluated, further sub-culturing of lyophilized bacteria will be determined by problems highlighted during the review of responses.
   - Vials are kept in the EQA laboratory until a review of responses and QC results are reconciled; can then be archived for further use as required.
4. Failure of QC:
   - Need 100% compliance.
   - Any non-viability of pathogen with bottles 1, 2, and 3 constitutes failure (mixed cultures – need to evaluate predominance of pathogen if appropriate to the specimen).
   - Hold shipment, repeat test with a larger number of vials (dependent on shipment size).
   - If repeat test consistent with the first non-compliance, stop shipment, evaluate necessary corrective action and start from the beginning of the procedure.
   - Subsequent vials are tested for viability over time; non-viability will determine the inability to evaluate laboratories with no growth.

WORKING CARD

QUALITY CONTROL FOR LYOPHILIZED SPECIMENS

Survey no: _____
Organism: _____
Preparation date:_____

1. Sterility QC (on non-selective media)

Sample	24 h	48 h
Skim milk obtained from DMP		

2. Contamination QC (after addition of organism)

Sample with organism	24 h	48 h
Before dispensing		
After dispensing		

3. Viability and contamination QC of organism after lyophilization

Number of samples depends on batch prepared; initial cluster needs to be sub-cultured, and then one sample per week for duration of shipment

Sample no.	Date sub-cultured	Date read	All media incubated for 24 h at 37 °C		
			Blood agar	Mac	Other
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					

Procedure WHO 0006	**PACKAGING OF EQA SAMPLES**		
Prepared by	**Date adopted**	**Signature**	
Vivian Fensham/ Anne von Gottberg			

Revision date	**Revision signature**	**Review date**	**Review signature**

Distributed to	**Number of copies**	**Distributed to**	**Number of copies**
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Purpose:

All samples that have been prepared for the EQA programme need to be labelled and packaged for shipment via courier.

Labelling procedure:

- The labels for the specimens are made using a label template. The labels have the survey number and the specimen number printed on them, e.g. **Survey 2005 1A.**
- All specimens are first labelled with their respective label before packaging.
- Each specimen is stored separately in its own preparation box.
- The exact same number of samples is prepared for each specimen number. This helps in the packaging procedure to prevent one laboratory getting duplicate specimens. A preparation box with an extra specimen means there has been a packaging mistake, which can be corrected before the survey is shipped.
- Slides are labelled with their respective survey and specimen number. This is done by manually writing on the slide with a lead pencil. The slides are then labelled (if necessary) with a small label with the respective survey and specimen information.

Packaging procedure:

- Bijou bottles containing the simulated stool specimens in Cary–Blair are each first packed in a universal container with a flow seal screw cap containing absorbent material.
- TI medium bottle tops are first sealed with parafilm and then each bottle is bubble-wrapped individually and sealed with tape.
- All slides are packed into slide mailers.
- Each slide mailer is labelled with the clinical information, e.g. malaria microscopy or TB microscopy (stained or unstained), etc.

- Sera for plague serology are dispensed in plastic screw top tubes (1.5 ml). These vials are then also placed in universal containers with absorbent material.
- All the specimens are then individually packed into a DHL Express watertight container with screw-lid and absorbent material as stipulated by IATA Regulations for diagnostic specimens.
- The watertight container is then packed into a DHL Diagnostic specimen box. This is in compliance with IATA regulations for PI650 (infectious substances in category B). The box is sealed and all the necessary documentation such as report forms and feedback forms are placed in a window envelope which is then attached to the outside of the box.
- The box and documentation is then placed inside a self-seal DHL express flyer bag with the airway bill and commercial invoice documents in the outside pouch of the bag.
- If the shipment is classed as “Infectious substances category A” then additional documentation and packaging requirements have to be met. The shipment in this instance has to be packed under PI 602, UN 2814 and a shipper’s declaration for dangerous goods has to accompany the shipment.
- The person who signs the “Shippers Declaration for Dangerous Goods” has to have attended an IATA course either for dangerous goods packaging or infectious substances 6.2. This person has to do a refresher course every two years.

Procedure WHO 0007	**SIMULATED CEREBROSPINAL FLUID (CSF) SMEAR**

Prepared by	**Date adopted**	**Signature**
Vivian Fensham/ Anne von Gottberg		

Revision date	**Revision signature**	**Review date**	**Review signature**

Distributed to	**Number of copies**	**Distributed to**	**Number of copies**
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Purpose:

Polymorphonuclear leukocytes (PMNs) mixed with an organism to simulate the appearance of a cerebrospinal fluid specimen containing a pathogen.

PMN preparation:

1. Place 6 ml of Polymorphprep™ (AXIS-SHIELD, PoC AS, Oslo, Norway) into each sterile screw-capped centrifuge tube.
2. Carefully layer 6 ml of fresh EDTA-treated blood onto the Polymorphprep™ without mixing the two solutions.
3. Spin the tube for 35 min at 1600 revolutions per min (rpm).
4. After spinning, the layers are observed: there is a large serum layer, a very dense and fluffy looking lymphocyte layer, a whiteish layer with a very faint cloud of denser material in it (PMNs), next the reagent layer with a button of erythrocytes.
5. Each layer is carefully removed and placed in a separate tube.
6. Add 4 ml of minimum essential medium (MEM), which has been brought to room temperature, to the tube containing the PMNs.
7. The fluids are gently mixed and then spun at 2200–2300 rpm for 10 min.
8. Pour off the supernatant and add another 4 ml of MEM to the tube. Gently resuspend the pellet and spin again as in step 7.
9. Pour off the supernatant and dribble 1 ml of MEM into the tube. Using a sterile pipette gently resuspend the cells.
10. Place 10 µl of the PMN suspension onto a clean glass slide and stain to check the numbers of cells present – there should be 6–10 PMNs/field viewed under the x100 oil immersion objective.

Organism suspension preparation:

1. The day before the PMN preparation, two tubes of Mueller-Hinton broth are inoculated with a colony of the organism, and incubated overnight.
2. The next day the tubes containing the broth and the organism are centrifuged for 10 min at 3300 rpm, the supernatant is discarded and 6 ml of phosphate buffered saline (PBS) is added.
3. If the organism does not require growth in a broth, it can be plated onto blood agar (preparation on blood agar or in broth will depend on pathogen).
4. The organism from the plate can be emulsified directly into the 6 ml of PBS and the optical density of the suspension read.
5. An optical density of 0,3 at a wavelength of 570 nm is required for the suspension; PBS is used as the blank.
6. One smear is made of the suspension to assess the concentration of bacteria.

Method of making the smear:

1. Make a 1:5 dilution of PMNs and bacterial suspension (1 part bacteria to 4 parts PMNs); mix well using a Gilford pipette.
2. Pipette 10 µl onto a clean glass slide, heat-fix and stain.
3. Examine the slide microscopically to check the number of cells and organisms per x100 field; varying dilutions of PMNs and bacteria can be made to obtain the best picture.
4. A small stirrer bar must be added to keep the product stirring while making the slides so as to give an even distribution of cells and bacteria on all the slides.
5. Slides can be stored at 4 °C for up to 3 months.

Quality control:

1. A sterility check must be carried out on all media and reagents used in the preparation of the simulated CSF smear.
2. Timing of QC of the slides (approximately 100 slides are made; 80 slides are made for despatch; 10 additional are made as replacements and kept in the fridge; 10 slides are made for QC).
   - Five slides are used for immediate QC; two staff members should review all five slides for quality of the smear, characteristics of the PMNs and bacteria.
   - Additional slides are kept at room temperature; and one is stained and reviewed on a weekly basis.
   - Slides are kept in the EQA laboratory until a review of responses and QC results are reconciled.
3. Failure of QC:
   - Need 100% compliance: slides 1–10 need to demonstrate the characteristics required by the case.
   - If any slide fails, hold shipment, review type of failure.
   - If review exposes a shortcoming in the smear preparation, postpone shipment and start from the beginning of the procedure.

WORKING CARD

QUALITY CONTROL FOR SIMULATED CSF SMEARS

Survey no: _____
Organism used: _____
Optical density reading: _____
Final dilution used: _____
Date of slide preparation: _____

Sterility check

Media/reagent	24 h incubation	48 h incubation
Minimum essential media		
Polymorphprep™		
Mueller-Hinton		
PBS		

Evaluation of Gram-stained films

Need to evaluate: details of size, shape, staining characteristics of the bacterium after in Gram-stained films to ascertain how accurately the smear reflects what could be expected in a clinical CSF specimen.

Slide no.	Date	PMNs/hpf*	Bacteria/hpf	Appearance of PMNs	Appearance of bacteria
1					
2					
3					
4					
5					

*hpf, high power field

Once the above QC has been completed and passed:

- The remaining slides are reviewed over a period of 5 weeks (1 per week).

Slide no	Date	PMNs/hpf	Bacteria/hpf	Appearance of PMNs	Appearance of bacteria
6					
7					
8					
9					
10					

Procedure WHO 0008	**PROCESSING OF WHO/NICD EQA RESULTS**

Prepared by	**Date adopted**	**Signature**
Vivian Fensham/ Anne von Gottberg		

Revision date	**Revision signature**	**Review date**	**Review signature**

Distributed to	**Number of copies**	**Distributed to**	**Number of copies**
EQA Microbiology Lab.	1		

Purpose:

Systematic review of responses from participating laboratories.

Procedure:

1. an Excel spreadsheet is created for the each survey (summary of response)
2. date of arrival of packages in each country is recorded (DHL tracking)
3. responses come in via fax, e-mail and post
4. date of receipt of responses is recorded
5. responses are acknowledged; method of acknowledgment and date are recorded
6. responses are initially entered onto the Excel spreadsheet in predetermined categories.

TIG meets approximately two to three weeks after specified return date to discuss:

- range of responses
- issues/difficulties/problems highlighted by the responses
- appropriate action to review all problems
- categories for marking are proposed
- possible mark allocations are proposed
- non-responders are identified and method of follow-up is determined

7. response-entry into Excel is checked by a second person
8. all specialist laboratories review the original responses for any points that may not have been recognized during entry into the spreadsheet
9. the Excel spreadsheet and mark allocations are formalized and distributed for review.

TIG meets again:

- to review second draft of data collection and marking
- to discuss the allocation of marks to each participant and its justification
- each specialist laboratory is requested to write and submit a commentary on the summary of responses.

10. TIG meets for finalisation of responses; review of global commentary (containing anonymous statistics)
11. Subsequent changes need to be reviewed by all panel members
12. all final documents are prepared to be distributed at the time of the next shipment
13. Excel spreadsheets (non-anonymous results) are only distributed to the Technical Implementation Group and the Technical Advisory Group
14. global commentary (containing anonymous statistics) is distributed to all participating laboratories
15. all the results from the Excel spreadsheet are then transferred to an Access database
16. individualized evaluation reports for each laboratory are then generated from this database
17. all final documentation is translated into French
18. final and updated versions of all documentation are saved and distributed by the EQA coordinator; back-up copies are saved (discs or on CD); hard copies are printed and filed when appropriate.

Procedure WHO 0009	PLAGUE: BIPOLAR STAINING BACILLI IN BLOOD FILM		
Prepared by	**Date adopted**	**Signature**	
Lorraine Arntzen/John Frean			

Revision date	Revision signature	Review date	Review signature

Distributed to	Number of copies	Distributed to	Number of copies
EQA Microbiology Lab.			

Purpose:

To produce a blood film showing bipolar bacilli, consistent with a diagnosis of septicaemic plague.

Selection of pathogen:

1. *Yersinia pestis* isolates from stored clinical isolates or control organisms.
2. Isolate (with laboratory or control strain number, date received in specialist laboratory) is sourced and processed in specialist laboratory (NHLS Special Bacterial Pathogens Unit).
3. For safety reasons, independent confirmation of identity is not required.

Isolate preparation:

1. All work is done in a class 2 biosafety cabinet in the BSL-3 laboratory.
2. Subculture the isolate from semisolid storage medium onto 5 % horse blood agar and incubate at 37 ºC aerobically for 48 hours.
3. Observe for pure growth after incubation.
4. Inoculate with specific phage (CDC); incubate at 27 ºC and 37 ºC.
5. Inoculate specific carbohydrate test media; incubate at 37 ºC.
6. Check phage susceptibility and fermentation reactions to confirm identity.

Method:

1. Draw 5 ml venous blood from a physically healthy volunteer.
2. Inoculate with several loopfuls of freshly-cultured *Y. pestis* isolate.
3. Incubate for 24 hours in capped tube.
4. Mix blood by repeated inversion of capped tube.
5. Produce blood smears, allow to dry, fix by immersion in methanol for 1 minute.

6. Check smears microscopically for presence of Gram-negative, bipolar-staining coccobacilli by Gram and Wayson stains at x1000 magnification.
7. Produce at least 50 slides.
8. Store in a sealed container until packed in plastic slide mailers.

WORKING CARD

QUALITY CONTROL FOR BIPOLAR STAINING BACILLI IN BLOOD FILM

Ten slides are stained and checked as described above; all are required to show small Gram-negative coccobacilli, the majority also showing bipolar staining.

Slide number	Gram-negative coccobacilli		Bipolar staining	
	Yes	No	Yes	No
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Procedure WHO 0010	**PLAGUE: ANTI-F1 ANTIBODY-POSITIVE SERUM**	
Prepared by	**Date adopted**	**Signature**
Lorraine Arntzen/John Frean		

Revision date	**Revision signature**	**Review date**	**Review signature**

Distributed to	**Number of copies**	**Distributed to**	**Number of copies**
EQA Microbiology Lab.			

Purpose:

To produce for serological testing rabbit serum specimens with antibodies that mimic natural exposure to plague. The procedure is in accordance with the joint CDC-WHO *Laboratory Manual of Plague Diagnostic Tests*, 2000.

Procedure:

1. Animal ethics application must be approved by the NHLS Animal Ethics Committee.
2. All the animal work is done by a registered, experienced animal technician.
3. Test bleed two adult New Zealand white rabbits; test for antibodies to F1 by ELISA and passive haemagglutination/haemagglutination inhibition (PHA/HI).
4. Dissolve 5 mg of *Y. pestis* F1 antigen (obtained from CDC, Fort Collins) in 1 ml of Freund's incomplete adjuvant.
5. Inoculate the antigen/adjuvant mixture (0.25 ml) intramuscularly into four sites in the hind legs of each rabbit.
6. Test bleed (5 ml) the rabbits after 3 weeks and check antibody titre by ELISA and PHA/HI.
7. If the titres are sufficiently high (>1:1024), bleed rabbits and separate serum.
8. Dilute serum to appropriate titre, aliquot in 1.5 ml volumes and freeze at -20 °C.
9. Normal rabbit serum from an uninoculated rabbit is used as negative specimen.

WORKING CARD

QUALITY CONTROL OF PLAGUE ANTI-F1 SERA

Sera are kept at room temperature and 37 ºC for two weeks and retested weekly for duration of shipment. The positive titre should not drop significantly.

Week number	Titre after	
	room temperature storage	37 ºC incubation storage
1		
2		
3		
4		
5		
6		
7		
8		

Procedure WHO 0011	SIMULATED SPUTUM SMEAR (with or without bacterial pathogen present)	
Prepared by	**Date adopted**	**Signature**
Kerrigan McCarthy/Vivian Fensham		

Revision date	Revision signature	Review date	Review signature

Distributed to	Number of copies	Distributed to	Number of copies
EQA Microbiology Lab.	1		

Purpose:

To manufacture simulated sputum smears containing polymorphonuclear leukocytes (PMNs), epithelial cells and normal flora of the oral cavity, together with mucous strands. Smears can be used as part of a bacteriology survey or acid-fast bacilli (AFB) microscopy survey (AFB negative slides).

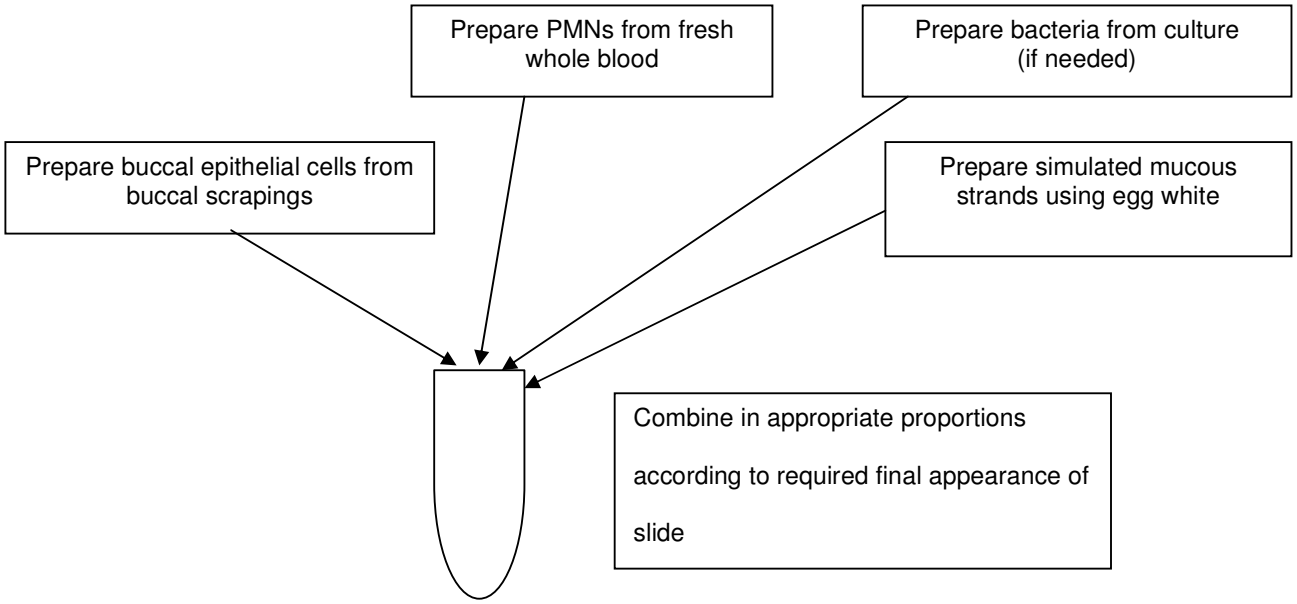
Reagents and consumables:

Polymorphprep™ (AXIS-SHIELD, PoC AS, Oslo, Norway)
15 ml fresh human blood collected in EDTA tubes
Minimum Essential Medium (MEM)
Ice
Phosphate buffered saline pH 7.2
One uncooked fresh egg
Bacteria in Mueller-Hinton broth
Sterile swabs
5 ml syringes
Hypodermic needles
Slides
15 centrifuge tubes
50 ml centrifuge tubes

Laboratory equipment:

Centrifuge
Magnetic stirrer
Vortex agitator

Overview of procedure:



PMN preparation (as in SOP WHO 0007):

1. Place 6 ml of Polymorphprep™ into each sterile screw-capped centrifuge tube; prepare three tubes to have sufficient cells.
2. Carefully layer 6 ml of fresh EDTA-treated blood onto the Polymorphprep™ without mixing the two solutions.
3. Spin the tubes for 35 min at 1600 revolutions per min (rpm).
4. After spinning, the layers are observed: there is a large serum layer, a very dense and fluffy looking lymphocyte layer, a whiteish layer with a very faint cloud of denser material in it (PMNs), next the reagent layer with a button of erythrocytes.
5. Each layer is carefully removed and placed in a separate tube.
6. Add 4 ml of MEM, which has been brought to room temperature, to the tube containing the PMNs.
7. The fluids are gently mixed and then spun at 2200–2300 rpm for 10 min.
8. Pour off the supernatant and add another 4 ml of MEM to the tube. Gently resuspend the pellet and spin again as in step 7.
9. Pour off the supernatant and dribble 1 ml of MEM into the tube. Using a sterile pipette gently resuspend the cells. This is the PMN mixture.
10. Place 10 µl of the cell suspension onto a clean glass slide and stain to check the number of cells present – there should be 6–10 PMNs/field viewed under the x100 oil immersion objective.

Preparation of epithelial cells:

1. Request two volunteers to rinse out their mouths with sterile phosphate-buffered saline (PBS), which they can spit into a basin. This is just to wash out the mouth prior to preparation.
2. Dip a sterile swab into PBS
3. Vigorously brush the buccal cavity of each volunteer with a swab.
4. Again rinse out the mouth with 10 ml PBS, and spit the mouth contents into a 50 ml conical centrifuge tube.
5. Rotate and press the sterile swabs against the sides of the 50 ml tube to release all the buccal epithelial cells into the PBS.
6. Vortex the tube (containing saliva, PBS and cells) vigorously for 10 sec.
7. Centrifuge the tube at 2200 rpm for 10 min.
8. Discard the supernatant.
9. Resuspend the pellet in PBS to make up 1 ml of solution.
10. Prepare a heat-fixed film of the suspension and Gram stain to confirm the presence of epithelial cells.

Organism suspension preparation:

(N.B. only required if simulated material is intended to represent sputum from a patient with a lower respiratory infection)

1. The day before the PMN preparation, two tubes of Mueller-Hinton broth are inoculated with a colony of the organism, and incubated overnight. If the organism does not require growth in a broth, it can be plated onto blood agar (preparation on blood agar or in broth will depend on pathogen).
2. The next day the tubes containing the organism are centrifuged for 10 min at 3300 rpm. The supernatant is discarded and 6 ml of PBS are added.
3. Adjust the optical density of the suspension to 0.3 (at 570 nm), using PBS as the blank.
4. The organism from the plate can be emulsified directly into the 6 ml of PBS and an optical density reading taken.
5. Make a smear of the suspension to assess the concentration of the bacteria.

Preparation of simulated mucus:

1. Separate the white of an uncooked egg
2. Make a 1:2 dilution of the egg white with PBS
3. This in then used as the background solution
4. Mix 1 ml of AFB smear fixative with 1 ml of the diluted egg white (only for AFB negative slides).

Method of making the smear:

1. Combine the PMN mixture with the epithelial cells and egg white background material in appropriate proportions.
2. Pipette 10 µl of the mixture onto a clean glass slide. The slide is heat-fixed and stained by Gram and Ziehl– Neelsen methods.
3. The slides are examined to check the number of cells and organisms per x100 field. Varying dilutions can be made to obtain the final acceptable dilution.

4. If bacteria are required, make a 1:5 dilution of PMNs and bacterial suspension (1 part bacteria to 4 parts PMNs) and mix well using a Gilford pipette.
5. Make a test slide to confirm that appearance is appropriate.
6. Use a magnetic stirrer to ensure even distribution of cells and bacteria on all the slides.

Quality control:

1. A sterility check must be carried out on all media and reagents used in the preparation of the simulated sputum smear.
2. If the slides are to be used in a TB microscopy survey, in addition to Gram's stain they should have Ziehl-Neelsen stain done to confirm the absence of mycobacteria.
3. The number of slides that are sent for quality control (QC) depends on the size of the batch that is to be made. On average 10% of the batch is quality controlled.
4. Ten slides are used for immediate QC: two staff members should review all 10 slides for quality of the smear, characteristics of the neutrophils and bacteria (if applicable);
    - If the slides prepared are for AFB microscopy panel testing and not simulated sputum smear, then 30 additional slides are sent externally for review: ten each to 3 different TB laboratories,– again each is asked to comment on the suitability of the slide as a simulated AFB smear and the characteristics of the neutrophils and bacteria;
    - any additional slides are kept at 4 ºC; and one is stained and reviewed on a weekly basis;
    - slides are kept in the EQA laboratory until a review of responses and QC results are reconciled.
5. Failure of QC:
    - need 100% compliance: slides 1–13 need to demonstrate the characteristics required by the clinical case;
    - if any slide fails, hold shipment, review type of failure;
    - if review exposes a shortcoming in the smear preparation, postpone shipment and start from the beginning of the procedure.

WORKING CARD

QUALITY CONTROL FOR SIMULATED SPUTUM SMEARS

Survey no: _____
Organism used: _____
Optical density reading: _____
Final dilution used: _____
Date of slide preparation: _____

Sterility check of media

Media/reagent	24 h incubation	48 h incubation
Minimum essential media		
Polymorphprep		
Mueller-Hinton		
PBS ph7.2		

Evaluation of slides

Need to evaluate: details of size, shape, staining characteristics of the bacterium after being Gram stained; and how accurately the smear reflects what could be expected in a clinical sputum specimen.

Slide no.	Date	PMNs/hpf*	Bacteria/hpf	Epithelial cells/lpf**	Appearance of PMNs	Appearance of bacteria	Background appearance
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

* hpf, high power field; ** lpf, low power field

Once the above QC has been completed and passed: Slides are sent to three separate clinical microbiology technologists/microbiologists for confirmation and clinical interpretation; separate report forms are completed, submitted and filed.

Additional slides to be examined on a weekly basis

Slide no	Date	PMNs/hpf*	Bacteria/hpf	Epithelial cells/lpf*	Appearance of PMNs	Appearance of bacteria	Background appearance
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							

* hpf, high power field; ** lpf, low power field

Annex 4B

Standard operating procedures for mycobacteriology EQA

Procedure WHO 0012	**MANAGEMENT OF AFB MICROSCOPY EXTERNAL QUALITY ASSESSMENT PROGRAMME**		
Prepared by	**Date adopted**	**Signature**	
Kerrigan McCarthy			

Revision date	**Revision signature**	**Review date**	**Review signature**

Distributed to	**Number of copies**	**Distributed to**	**Number of copies**
EQA Microbiology Lab.			

Objective:

The purpose of this SOP is to detail the management processes required to operate the WHO/NICD external quality scheme for acid fast bacilli microscopy (tuberculosis diagnostic). This SOP is based on WHO, IUATLD and CDC recommendations as published in '*External Quality Assessment for AFB Smear Microscopy*' published by the Association of Public Health Laboratories, September 2002.

Theory:

External Quality Assessment (referred to by the WHO as 'panel testing') is one of the recommended methods to determine whether a laboratory technician can adequately perform AFB smear microscopy. Other methods are blinded rechecking of diagnostic specimens and on-site evaluation of laboratory services. EQA is a minimal first step for laboratory services. It allows rapid assessment of gross deficiencies in a TB diagnostic service. It may also assist in identifying factors contributing to errors. It allows assessment of training of microscopists. Advantages and disadvantages of panel testing are shown below.

Panel testing of AFB smear microscopy	
Advantages	**Disadvantages**
• Will not impact on workload of a peripheral TB diagnostic laboratory • Improves laboratory credibility • Rapid evaluation from a large area is possible • Results can be used to identify source of problems leading to poor performance	• Does not measure routine laboratory performance • May not motivate staff to improve daily performance

Background:

The programme uses the IUATLD grading system for grading slides as in Table 1 below. The programme uses the Ziehl-Neelsen staining method exclusively. However, participating laboratories are free to choose staining method for unstained slides.

Table 1
IUATLD Grading system for TB microscopy slides

Carbol-fuchsin stain under oil (1000x)	Report
No AFB	No acid-fast bacilli observed
1 to 9 AFB/100 fields	Record exact figure (scanty)
10 to 99 AFB/100 fields	1+
1 to 10 AFB/field	2+
> 10 AFB/field	3+

AFB, acid-fast bacilli

Overview of EQA programme:

- Test smears are prepared by Microbiology EQA Unit according to SOP 0013.
- A proportion of the prepared batch are quality controlled according to SOP 0013.
- Three surveys are sent out to participating laboratories each year. Each survey comprises eight slides (of which four will be stained using the Ziehl-Neelsen method), an instruction sheet and response forms.
- Surveys are distributed through existing transport channels.
- Response forms and smears will be returned for evaluation to the NICD EQA Unit.
- Evaluation of responses is done according to this SOP.
- Reports detailing laboratory performance will be distributed to participating laboratories and appropriate authorities.
- Laboratories will be expected to implement corrective action following poor performance.

Procedure:

Preparation and quality control of smears

Refer to SOP 0013.

Number and type of smears

- Each test panel includes eight slides, of which four are stained according to the Ziehl-Neelsen method, and four are unstained.
- All laboratories receive identical test panels.
- The test panel includes slides with different grades of positivity in order to evaluate the ability of the technicians to properly grade positive slides.
- The composition of the test panels varies each survey, but the degree of difficulty is increasing.

Communication with laboratories

An instruction sheet for participating laboratories is found in Appendix 5 of this document. A response form for laboratories to record results is found in Appendix 5 of this document.

Time allowed for test laboratories to review panel and report results

Laboratories are expected to respond to challenges by the survey closing date, which shall generally be 4 weeks after shipment of panels. Late results are accepted, but laboratories are penalised.

Evaluation and interpretation of results

Results are entered into Access® TB microscopy database. Errors are classified automatically by the database programme according to Table 2. A score is allocated, according to Table 3 below. An acceptable score is 65 marks.

Table 2
Classification of errors

Result of participating laboratory	Result as deemed by EQA Unit				
	Negative	1-9 AFB/100fields	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
1-9 AFB/100 fields	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

Interpretation:

QE = Quantification error	Minor error
LFN = Low false negative	Minor error
LFP = Low false positive	Minor error
HFN = High false negative	**Major error**
HFP = High false positive	**Major error**

Table 3
Scoring system

Classification	Interpretation	Score
Correct response	No error	10 points
QE	Minor error	10 points
LFN, LFP	Minor error	5 points
HFN, HFP	Major error	0 points

Feedback

Each participating laboratory will receive reports that include both individual results as well as aggregate performance for all laboratories tested. Examples of reports are found in Appendix 5 of this document.

Responsibility:

Overall responsibility for the AFB microscopy EQA programme shall rest with the Head of the EQA Unit. The senior technologist in the EQA Unit shall be responsible for preparing slides, finalizing accompanying documentation, data entry of responses and checking of reports. Reports shall be checked by the Head of the EQA Unit prior to posting. The database shall be maintained by the Head of EQA Unit. Posting and packaging of surveys

and reports shall be done by the organizer. Discrepancies shall be checked by the senior technologist in the EQA Unit and a microscopist in the NHLS Central Tuberculosis Laboratory.

Procedure WHO 0013	PREPARATION OF POSITIVE REFERENCE SMEARS FOR INCLUSION IN THE WHO/NICD TUBERCULOSIS MICROSCOPY EXTERNAL QUALITY ASSESSMENT PROGRAMME

Prepared by	Date adopted	Signature

Revision date	Revision signature	Review date	Review signature

Distributed to	Number of copies	Distributed to	Number of copies
EQA Microbiology Lab.			

Objective:

The purpose of this SOP is to describe the procedures necessary to prepare and quality assure reference smears for the NICD tuberculosis (TB) microscopy EQA programme.

Theory:

The role of external quality assessment in TB microscopy quality assurance is described in SOP 0012. Preparation of reference material of consistent quality and grade is integral to the efficient running of a TB EQA programme. This method has been adapted from WHO, IUATLD and CDC recommendations as published in '*External Quality Assessment for AFB Smear Microscopy*' published by the Association of Public Health Laboratories, September 2002.

Procedure:

Materials required:

50 ml plastic centrifuge tubes
4% sodium hydroxide (NaOH), sterile
Vortex
Water-bath at 55–60 ^{o}C
Phosphate buffer saline (PBS) pH 6.8
Centrifuge
Microscope slides
Reagents for acid-fast stain (carbol-fuchsin, ethylene blue and 3% acid alcohol)
Graduated sterile plastic disposable pipettes
20 µl and 1000 µl micropipettes, yellow and blue pipette tips
1% Hycolin disinfectant

AFB positive reference material:
The NHLS TB Laboratory, Central Gauteng, provides the EQA Unit with an appropriate volume of concentrated, heat-sterilized sputum that has been pooled from specimens graded 3+ according to the IUATLD (International Union against Tuberculosis and Lung Diseases) staging.

AFB negative reference material:
The NHLS TB Laboratory, Central Gauteng, provides the EQA Unit with an appropriate volume of concentrated, heat-sterilized sputum that has been pooled from specimens in which no AFB have been observed.

Preparation of AFB positive stock:

- label 50 ml plastic centrifuge tubes from 1–10 as '**Positive AFB'**;
- decant 1 ml of the AFB positive reference material into each labelled 50 ml tubes;
- add 1 ml of 4% NaOH to each tube, so that the final concentration of NaOH is 2%;
- vortex each tube thoroughly for 4–5 min;
- add up to 20 ml of PBS pH 6.8 to each tube and mix well;
- incubate all tubes in a water-bath (preferably with a shaker) for 30 min at 55–60 ^{o}C; if no shaking water-bath is available then mix occasionally by inverting tubes;
- make up the volume in each tube to 40 ml by adding phosphate buffer pH 6.8;
- centrifuge at 3000 rpm for 20 min at room temperature;
- decant the supernatant carefully; resuspend pellets in 0.5–1 ml of PBS pH 6.8; combine the pellets from all tubes into one centrifuge tube labelled 'AFB positive stock'; vortex for 30 s to mix pellets thoroughly.

Preparation of AFB negative stock:

- label 50 ml plastic centrifuge tubes from 1–10 as **'Negative AFB'**;
- decant 1 ml of the AFB negative reference material into labelled 50 ml tubes;
- add 1 ml of 4% NaOH to each tube, so that the final concentration of NaOH is 2%;
- vortex each tube thoroughly for 4–5 min;
- add up to 20 ml of PBS pH 6.8 to each tube and mix well;
- incubate all tubes in a water-bath (preferably with a shaker) for 30 min at 55–60 ^{o}C, if no shaking water-bath is available, then mix occasionally by inverting tubes;
- make up the volume in each tube to 40 ml by adding PBS pH 6.8;
- centrifuge at 3000 rpm for 20 min at room temperature;
- decant the supernatant carefully; resuspend pellets in 0.5–1 ml of PBS pH 6.8; combine the pellets from all tubes into one centrifuge tube labelled 'AFB negative stock'; vortex for 30 s to mix pellets thoroughly.

Evaluation of positive and negative stock preparations:

- label slides using pencil 'AFB positive stock' and 'AFB negative stock';
- using a standard microbiological loop (0.001 ml) make 2–3 tests smears from both positive and negative stock;
- dry and fix the smears;

- stain the smears using the Ziehl-Neelsen stain;
- perform microscopy under 100x oil immersion;
- optimal smear preparation should result in 50–60 AFB per microscope field in the positive stock and none in the negative stock;
- record the number of AFB per field on the reference stock tube.

How to prepare background material for TB slides before adding positive stock (refer to SOP WHO 0011)

To make up 2ml of background material requires:

1.0 ml negative stock
0.5 ml AFB fixative
0.5 ml 1:2 dilution of egg white in PBS
Epithelial cells and neutrophils

Dilution procedure:

Dilute the positive stock with the background material stock to reduce the number of AFB per microscope field or per 100 fields to within the required range. Table 1 below suggests the number of AFB that should be aimed for to fall within the IUATLD range.

Table 1
IUATLD quantification, and intended AFB quantification of reference material

IUATLD grade	IUATLD quantification	Quantification of reference material
Negative	0 AFB/100 fields	No AFB
Exact number	1–9 AFB/100 fields	5 AFB/100 fields
1+	10–99 AFB/100 fields	50 AFB/100 fields
2+	1–10 AFB/field	5 AFB/field
3+	> 10 AFB/field	20 AFB/field

For preparation of 3+ quantification:

- Initially make a 1:2 dilution with background material and positive stock. Prepare a few slides for staining and count the number of AFB per field.
- Calibrate an ordinary Pasteur pipette to find out how many drops are present in 1 ml of stock material (usually 19–20 drops make up 1 ml).
- Use the following formula to calculate how many drops of positive sputum to be added.

$$\text{No of drops of positive sputum to be added (N)} = \left(\frac{\text{Desired AFB concentration (DC)}}{\text{Actual AFB concentration (AC)}}\right) * \text{Amount of drops in required volume of sputum (A)}$$

The above formula and dilution procedure applies to the preparation of 1+ and 2+ positive smears.

Example 1:
Prepare 5 ml suspension to make slides with 2+ quantification

Establish the facts:

- AFB concentration in the stock suspension (AC) is 65 AFB/field
- 5 ml of suspension (A) contains 75 drops (1ml was calibrated to contain 15 drops)
- 2+ suspensions should have an AFB concentration (DC) of 5 AFB/100 fields.

Complete the formula:

$$\text{No of drops of positive sputum to be added (N)} = \frac{5 \text{ AFB}}{65 \text{ AFB}} * 75 \text{ drops}$$
$$= 5.7 \text{ drops}$$
$$= 6 \text{ drops}$$

Using the Pasteur pipette, take 6 drops of positive stock preparation, and mix it with 69 (75–6) drops of background stock preparation. Mix the solution thoroughly using the vortex mixer. Label the tube '2+ stock solution', and use immediately to prepare slides.

Example 2:
Prepare 5 ml suspension to make slides with scanty/exact number/100 fields (i.e. lowest possible positive quantification)

Establish the facts:

- AFB concentration in the stock suspension (AC) is 65 AFB/field,
- 5 ml of suspension (A) contains 75 drops (1 ml was calibrated to contain 15 drops)
- Exact number/100 fields suspension should have an AFB concentration (DC) of 5 AFB/field.

Complete the formula:

$$\text{No of drops of positive sputum to be added (N)} = \frac{5 \text{ AFB/100 fields}}{6500 \text{ AFB/100 fields}} * 75 \text{ drops}$$
$$= 0.06 \text{ drops}$$

If 1ml contains 15 drops, then 1 drop = 1/15 ml, i.e. 1 drop = 0.07 ml
0.06 of a drop = 0.07*0.06 ml
= 0.004 ml
= 4 µl

Using the Eppendorf pipette, dispense 4 µl of positive stock preparation, and mix it with 75 drops of negative stock preparation. Mix the solution thoroughly using the vortex mixer. Label the tube 'scanty stock solution', and use immediately to prepare slides.

Preparation of slides

- place the stock solution on a magnetic stirrer
- prepare and label the required number of batches of slides

- using a standard graduated micropipette and Eppendorf tip, transfer 10 µl of material onto the slide to make a diameter of 2 centimeters.
- heat fix the slides on a slide dryer.

Internal quality control to validate AFB smears

- randomly select ten slides (between 5 and 10%) of the batch;
- label the slides numerically (e.g. 1 to 10);
- stain the slides using the Ziehl-Neelsen (ZN) procedure;
- complete the log form in appendix (of this SOP), which shall be recorded on the spreadsheet; label the batch according to the date it was made, and the batch serial number (e.g. 280204-1 for the first batch ever); list the number of slides made and the date the slides were made;
- perform microscopy under oil immersion on the slides; record the results in the log sheet;
- request another microscopist, blinded to the results, to perform microscopy on the same slide set;
- acceptable results: calculate the standard deviation (SD) and the consistency according to the following formula:

$$SD = \sqrt{\frac{\left(x - \overline{x}\right)^2}{(n-1)}}$$ Consistency = Mean – (SD*2)

- interpret the result in the consistency column using the following formula
  M – 2SD
  If M-2SD > 0, then consistency is sufficient
  If M-2SD < 0, then consistency is insufficient.

External quality control to validate AFB smears (random check)

- randomly select ten slides from two batches (i.e. 5 of batch A and 5 of batch C);
- both stained and unstained slides are labelled numerically and are sent to 3 referee laboratories with relevant documentation;
- the above procedure is performed a week before the TB Microscopy programme goes out;
- results are then compiled on a "TB Micro result form" and evaluated; if acceptable the programme is based on acceptable Quality Control (QC), minimally if QC failed the batch is then discarded;
- acceptable results: calculate the standard deviation (SD) and the consistency according to the following formula:

$$SD = \sqrt{\frac{\left(x - \overline{x}\right)^2}{(n-1)}}$$ Consistency = Mean – (SD*2)

- interpret the result in the consistency column using the following formula
  M – 2SD
  If M-2SD > 0, then consistency is sufficient
  If M-2SD < 0, then consistency is insufficient.

Appendix
Validation log for AFB panel testing slide batches

Batch	Slide preparation			Slide evaluation													
	No. slides made	Date slides made	Int[a] result	Slide test results (AFB per 100 fields) 1	2	3	4	5	6	7	8	9	10	Mean	SD[b]	Cons[c]	Final result
*	225	09/09	2+	320	360	410	420	410	460	340	380	330	240	367	63.08	240.84	Acc[d]

* example of use of table
[a] intended result
[b] standard deviation
[c] consistency
[d] acceptable

Annex 4C

Standard operating procedures for malaria EQA

Procedure WHO 0014	SELECTION AND PREPARATION OF BLOOD SMEARS FOR MALARIA EQA		
Prepared by	**Date adopted**	**Signature**	
Leigh Dini/ John Frean			

Revision date	Revision signature	Review date	Review signature

Distributed to	Number of copies	Distributed to	Number of copies
EQA Microbiology Lab.			

Objective

This SOP describes the selection of appropriate malaria positive blood to be used for parasitology external quality assessment (EQA) surveys. It also describes the preparation of blood smears.

Principle

Good quality, clinically-relevant blood specimens are selected and prepared for EQA surveys that test a participant's quality.

Background and introduction

Malaria specimens are selected for inclusion in an EQA survey based on certain criteria. These include quality of the specimen, malaria species and load (or density) of parasites present and type of challenge required. After selection the specimens are prepared in a suitable form for distribution to participants. A number of quality control steps are performed during selection and preparation of EQA material. A list of available EQA material with specimen and sender's details is kept in a file.

Responsibility

The medical technologist or scientist is responsible for selection and preparation of EQA material.

Specimen collection and handling

EQA material is sourced from routine specimens and volunteers. Specimens should be treated as infectious at all times. EDTA-treated blood specimens should ideally be less than 4

hours old. Specimens older than 8 hours are not suitable. At least 2ml of EDTA blood is required.

Sample type

EDTA-treated blood

Equipment and materials

70% alcohol pad

Vacutainer or syringe with needle (21 or 23 gauge)

EDTA-filled tube

Gloves

Glass slides

Pasteur pipettes

Binocular microscope with x10, x40, x50 and x100 objectives

Micropipette 2–20µl (separate micropipettes are used for negative specimens to avoid contamination from positive specimens)

Pipette tips

Photocopied templates for thick and thin blood smears

Tub

50–60 °C hotplate

Dishwashing liquid

Gauze or lint-free towel

Slide boxes

Desiccant, namely silica gel

Quality control

Quality control steps are included in the procedure for selection and preparation of EQA material.

Safety precautions

Universal precautions should be used when handling blood, including wearing gloves and protecting the eyes.

Procedure

I. Selection of EQA material

Blood specimens are selected on the following basis:

- Freshness and quality of specimen: if routine EDTA-treated blood is used, blood specimens should be fresh, i.e. less than 8 hours old (ideally less than 4 hours old). Use one donor per batch of blood slides prepared.
- Load or density of parasites present: in most cases the final concentration of parasites in the challenge should be such that a technologist with average skill and experience can detect their presence in a reasonable amount of time.
- Quality of parasites present: parasites should be easily recognizable with few abnormal or degenerative forms present. Patient's should be sampled prior to treatment.
- Species of parasite present: the type of challenge for the current survey and a summary of past challenges assist in determining the type of parasites required for the current survey.
- Appropriateness for the challenge: specimens should be chosen to suit the objectives of the current challenge.

II. Consent of donors and phlebotomy

This is required for non-routine specimens sent for malaria diagnosis.

1. Obtain informed consent from donor using the attached Appendix A.
2. Follow standard procedure for phlebotomy of an antecubital vein.
3. Clean the venipuncture site with an alcohol pad and allow to air dry.
4. Use a sterile non-reusable phlebotomy needle. Draw 2.5–3ml of blood into an EDTA-filled tube and gently mix by inverting the tube.
5. Use only one donor per batch of smears and prepare smears soon after blood collection (within 4 hours).

III. Procedure for cleaning slides

1. Open a new package of glass slides.
2. Place slides in a tub of soapy water, with standard dishwashing liquid, and soak for at least 2 hours.
3. Rinse slides well with tap water.
4. Thoroughly dry each slide with gauze or lint-free towel.
5. Leave on a 50–60°C hotplate to dry completely.
6. Store slides in a slide box with silica gel.

IV. Preparation of blood smears

1. See Appendix B for a flowchart of this procedure. Complete a specimen preparation work card (see Appendix C) for each EQA specimen. Attach a copy of the patient's laboratory report to the work card.
2. Photocopy paper templates for thick smears (see Appendix D) and thin smears (see Appendix E).
3. Place glass slides on top of paper templates and label with a "C" on the frosted area. Lay out 200 slides for thin smears and 200 for thick smears. Use gloves when handling slides to avoid traces of oil from the fingers.
4. Assess the volume of blood in the EDTA tube. About 2 ml is required to produce 200 thick and 200 thin smears, including discarded poor quality smears.
5. Mix the blood regularly during slide preparation to ensure uniform dispersal of parasites. Use a micropipette to deliver 2 µl of blood per thin smear onto the centre of the line of the template. Immediately perform a thin smear using a spreader, before any

drying occurs. Check for a good quality feathered edge and discard poor quality smears (i.e. uneven smears, ragged edges, presence of bubbles, clots, streaks or other defects).

6. Use a micropipette to deliver 6 µl of blood per thick smear. Place the drop in the centre of the circle of the template. Using a spreader, spread the blood outwards to form an even circle of 12 mm diameter. If bubbles are present break them with the sharp corner of another slide. Discard poor quality smears, i.e. uneven smears, presence of bubbles, clots, streaks or other defects.
7. Discard the pipette tips and spreaders regularly when clots start to form and the smear quality deteriorates.
8. Leave thin smears to dry horizontally for at least 30 minutes, and then check for good quality feathered edges. Fix thin smears in 100% methanol for 5 seconds and allow to air dry completely. Leave thick smears horizontally to dry overnight and do not fix them. Do not apply any rapid heat to dry the smears. Protect smears from dust and insects until they are stored.
9. Stain smears with Giemsa (see SOP number WHO 0015).
10. Store stained slides in slide boxes and label with the patient's name and hospital number.
11. Discard the paper templates and spreaders, as re-used templates and spreaders will contaminate the next batch of slides.

Bibliography

Hydas Inc/NAMRU-2. 2005. Creation of validated blood smears for the microscopic diagnosis of malaria. Hydas Inc. Pennsylvania USA and NAMRU-2 Jakarta Indonesia. Supported by NIAID contract NO1-AI-85355 (MR4).

Procedure WHO 0014	**SELECTION AND PREPARATION OF BLOOD SMEARS FOR EQA**
APPENDIX A:	**Informed consent form for donors**

Information:

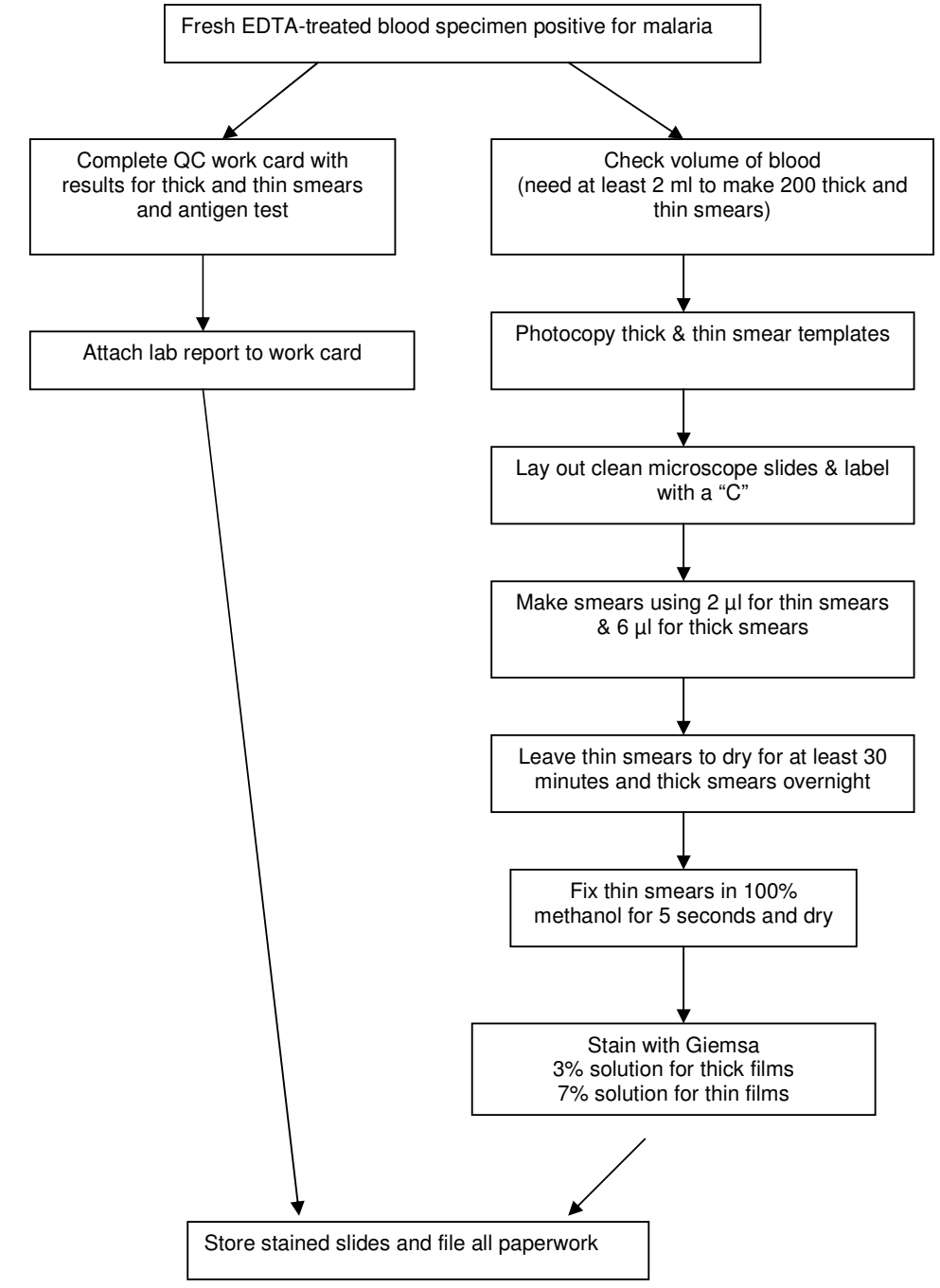
Hello. My name is__(name of phlebotomist)__and I would like to ask you if I can take a 3 ml (less than 1 teaspoon) blood sample from your vein. We would like to make blood smears with your blood for teaching purposes. This is completely voluntary. If you decide not to volunteer it will not affect your treatment in any way. Please feel free to ask me any questions. Thank you for your time. There is a form below that you need to sign if you agree to us taking a blood sample.

Consent given for blood sample:

Donor's name & signature:	Date:
Witness's name & signature:	Date:

Interviewer's name & signature:_____

Procedure WHO 0014	**SELECTION AND PREPARATION OF BLOOD SMEARS FOR EQA**
APPENDIX B:	**Preparation of blood smears for EQA**



Procedure WHO 0014	**SELECTION AND PREPARATION OF BLOOD SMEARS FOR EQA**
APPENDIX C:	**EQA Programme Specimen preparation work card**

Attach a copy of the laboratory report.

Blood parasites present (species and stages observed):_____

_____

_____

Parasite count: _____% or parasites/µl

P. falciparum antigen test result: positive ☐ negative ☐

Patient's history:_____

_____

_____

Number of thick smears made: 200 Other: _____

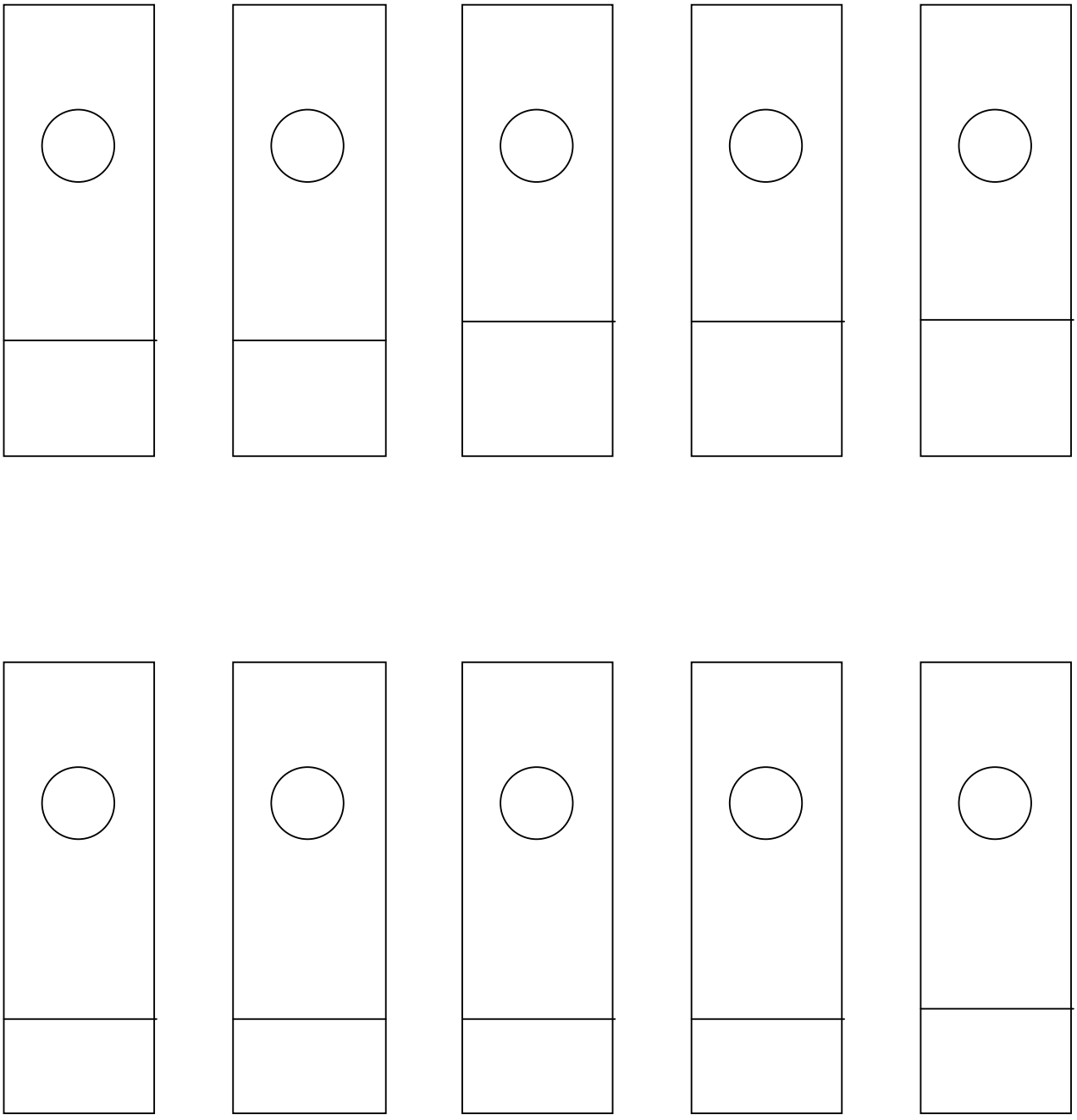
Number of thin smears made: 200 Other: _____

Technologist/s who prepared slides:_____

Signed:_____ Date:_____

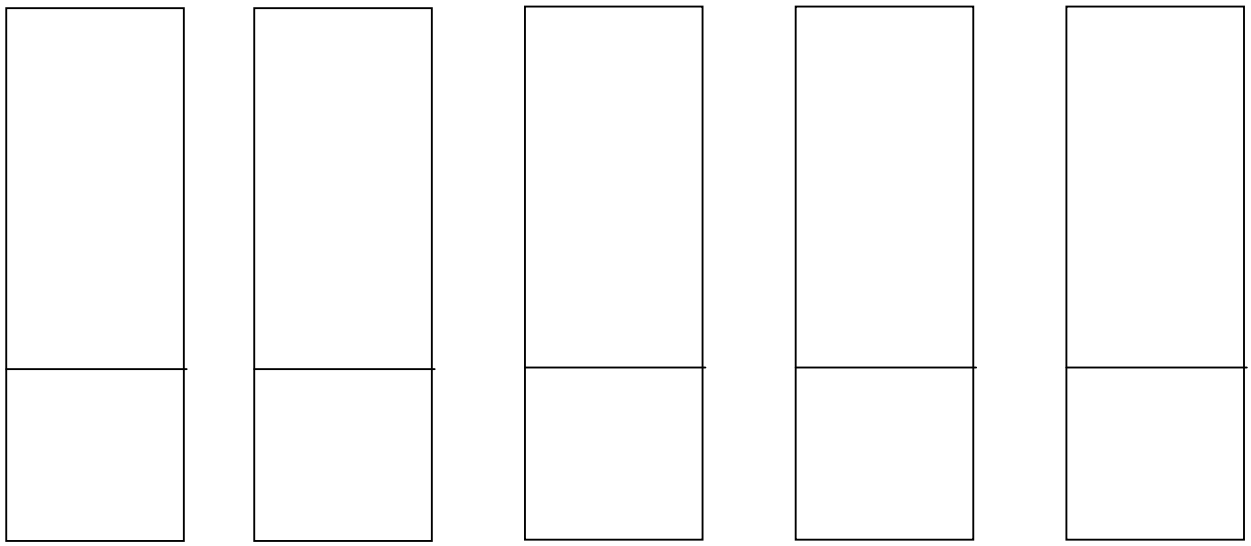
Procedure WHO 0014	SELECTION AND PREPARATION OF BLOOD SMEARS FOR EQA
APPENDIX D:	Thick blood smear template

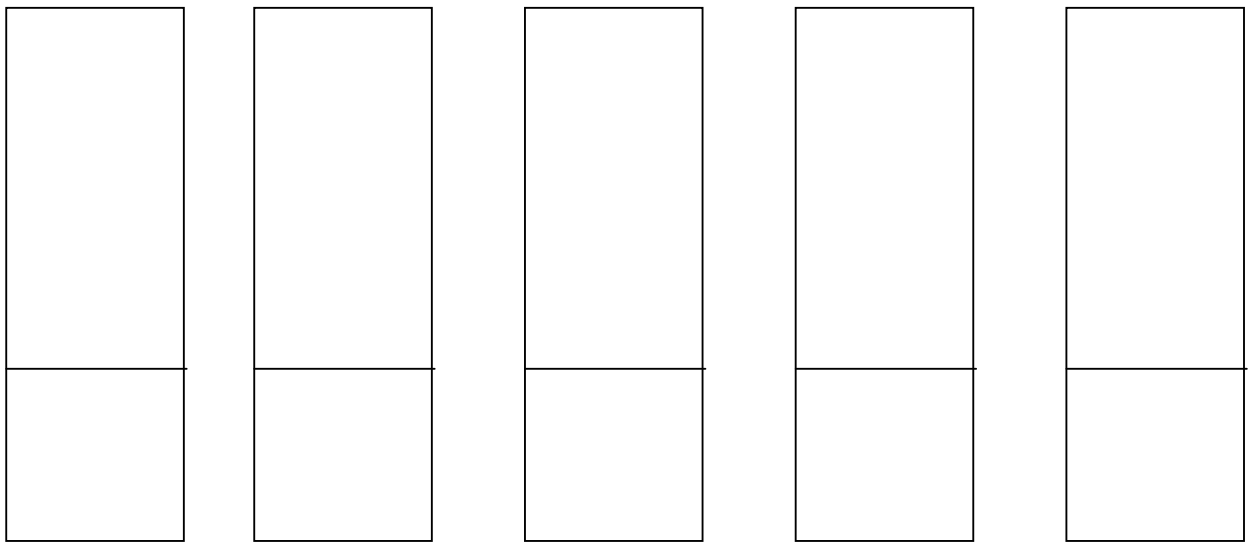
Thick blood smear template; use 6 µl of blood (diameter of circle is 12 mm)



Procedure WHO 0014	SELECTION AND PREPARATION OF BLOOD SMEARS FOR EQA
APPENDIX E:	Thin blood smear template

Thin blood smear template; use 2 µl of blood





Procedure WHO 0015	MASS GIEMSA-STAINING OF BLOOD FILMS FOR EQA	
Prepared by	**Date adopted**	**Signature**
Leigh Dini and Rita van Deventer		

Revision date	Revision signature	Review date	Review signature

Distributed to	Number of copies	Distributed to	Number of copies
EQA Microbiology Lab.			

Objective

This SOP describes the method used to stain a large number of blood smears at the same time with Giemsa for external quality assessment (EQA) purposes.

Principle

Well-stained blood smears are required for EQA purposes. This is a method to stain a large number of slides with Giemsa at the same time.

Responsibility

The medical technologist is responsible for staining and assessing the quality of slides.

Safety precautions

Gloves should be worn throughout the procedure. Methanol and Giemsa stain are highly flammable and toxic if inhaled or swallowed.

Specimen type, collection and handling

Specimen type: thick and thin blood smears on glass slides.
Thin blood smears should be fixed within a few days of being produced.
All smears should be stained within 10 days of being produced.

Equipment and material

Giemsa stain
Sorensen's phosphate buffer pH 7.2
Distilled water
Staining racks and containers
Whatman no 1 filter paper
Measuring cylinder
Beaker
Funnel
Rubber tubing
Fan

Procedure

1. Check that all slides have a "C" marked on them to indicate the right side up. Discard poor quality smears (e.g. no feathered edge on thin smears, damaged slides or smears). Be careful not to scratch the blood films during handling.
2. Stain one patient's slides per batch. Use separate staining racks and containers for parasite-negative blood smears.
3. Place thick and thin smears in separate staining racks.
4. Fix the thin smears in 100% fresh methanol for 5 seconds and allow to air-dry. Do not fix the thick smears.
5. Calculate the volume of Giemsa solution needed for the batch of slides to be stained (refer to Appendix).
6. Dilute 1 part of Sorensen's phosphate buffer (pH 7.2) to 2 parts of distilled water.
7. Using this diluted buffer make a 3% Giemsa solution for thick films and a 7% Giemsa solution for thin films.
8. Filter the 7% Giemsa solution for thin films through a Whatman no1 filter paper before use.
9. The Giemsa solution for thick films need not be filtered.
10. Place the staining rack with slides into a staining container in the sink, and gently cover slides with the appropriate Giemsa solution. Leave to stain for 45 minutes.
11. Insert the end of a rubber tube attached to cold water tap into the bottom of the staining container and gently open the tap to float the iridescent scum off the surface of the stain. The water speed can gradually be increased to help the rinsing process.
12. Continue rinsing until the water from the container runs clear.
13. Allow the slides to air-dry completely in front of a fan.
14. Check a thick and thin smear from every stain batch under the microscope, to ensure stain quality is acceptable. Blood cells on thick smears should be lysed, white blood cell nuclei should be dark purple and red blood cells should be light grey in colour.
15. Discard poor quality slides that have been damaged during staining.
16. Place the slides in slide trays, date and label them, and enter the details into a file.
17. Store the slide trays in the glass cupboards.

Potential source of variability

Giemsa stock varies in quality and concentration. Each new batch should be checked for stain quality before use.

Internal Quality Control

A thick and thin smear from every stain batch is checked microscopically to ensure stain quality is acceptable.

Bibliography

World Health Organization. 2000. Bench aids for the diagnosis of malaria infections. 2nd edition. WHO Geneva.
John Storey. 2005. Personal communication.

Procedure WHO 0015	**MASS GIEMSA-STAINING OF BLOOD FILMS FOR EQA**
APPENDIX	**Example of calculations for Giemsa staining**

Giemsa staining is performed in a square 3 litre staining container. The staining rack holds 100 slides.

First make up 1500 ml of diluted buffer: 1000 ml distilled water + 500 ml Sorensen's buffer

Thin films:
Make a 7% Giemsa solution: 105 ml Giemsa stock stain made up to 1500 ml with diluted buffer.

Thick films:
Make a 3% Giemsa solution: 45 ml Giemsa stock stain made up to 1500 ml with diluted buffer.

Procedure WHO 0016	QUANTITATION OF MALARIA PARASITAEMIA		
Prepared by	**Date adopted**	**Signature**	
Leigh Dini & John Frean			

Revision date	Revision signature	Review date	Review signature

Distributed to	Number of copies	Distributed to	Number of copies
EQA Microbiology Lab.	1		

Objective

This method describes how to quantitate the parasitaemia of *Plasmodium falciparum* in a patient's blood.

Responsibility

The technologist is responsible for performing the parasite count and reporting of results.

Background and introduction

Quantitation of malaria parasitaemia gives an indication of parasite load and correlates approximately with severity of clinical condition. A parasite count is performed after parasite identification has confirmed the presence of *P. falciparum* parasites. Two methods are used to calculate parasitaemia:

Method 1: Only red blood cells containing trophozoite stages are counted, not ones containing schizonts or gametocytes. Red blood cells are counted in an area of the thin blood film close to the feathered edge, where cells are evenly distributed and not overlapping.

Method 2: Count the number of parasites against leukocytes in a thick film and, using either the actual leukocyte count or a standardized count, express the load as parasites per microlitre of blood.

Note: The semi-quantitative "plus" method is only appropriate for routine field microscopy where the decision to treat or not to treat is in question. It is not appropriate for reference microscopy, trials, treatment monitoring or determination of higher levels of microscopy competence.

Principle

Two methods are used to calculate parasitaemia: (1) the percentage of parasitised red blood cells in a representative sample of a stained thin blood film is determined using oil-immersion microscopy, and (2) count parasites against leukocytes on a thick blood film and express it as parasites per microlitre of blood.

Frequency of testing

This test is performed on request, as part of routine laboratory diagnosis of malaria.

Sample type

Giemsa-stained thick and thin blood smears with *Plasmodium falciparum* malaria parasites

Equipment and materials

Binocular microscope with x100 oil immersion objective
Miller squares eyepiece graticule
Giemsa-stained thin blood smear
Counters

Calibration

The microscope should have been serviced within the last 12 months.

Procedure

Method 1: Parasite count on a thin blood smear

1. Using a Miller squares eyepiece graticule, count the number of red blood cells in the small square and multiply this by 10 to obtain an estimate of the total number of red blood cells in the large square. Do not count cells that touch 2 adjacent sides of the small square, as for urine or CSF cell counts.

2. Count the number of infected red blood cells in the large square, including the small square. Do not count cells that touch 2 adjacent sides of the large square. Count infected red blood cells, not parasites.

3. Move to the next field and repeat. Continue until the red blood cells total to at least 1000.

4. Add up the infected red blood cells and work out percent parasitaemia using this formula: (total number of infected red blood cells ÷ total number of red blood cells counted) by 100 = percentage parasitaemia.

5. To estimate the number of parasites per µl from a thin smear, use this formula: (total number of infected red blood cells ÷ total number of red blood cells counted) x $5X10^6$ (or the patient's actual erythrocyte count per µl, if available) = number of parasites per µl.

6. If a Miller squares graticule is not available, an accurate estimate of the number of red blood cells per field has to be made. It helps to divide the field into quadrants by imaginary lines and estimate cells per quadrant. Then count the number of infected red

blood cells in the whole field.

Method 2: Parasite count on a thick blood smear
Determination of parasites/µl of blood is accomplished by counting the number of parasites on a thick film in relation to the patient's actual number of white blood cells (WBCs) per µl of blood. If the patient's WBC count is not available then an assumed average number of 8000 is used, however this is less accurate. Three tally counters are required, one for counting WBC, one for counting asexual forms and one for gametocytes.

1. Before starting the count, complete a thorough examination of the thick and thin films to identify any parasites present.

2. On the thick film find a field with 12 WBCs or more and start the count here.

3. Separately count the number of sexual and asexual parasites until reaching the field with the 200th WBC (many experts believe that a minimum of 500 WBCs should rather be counted to be more accurate).

4. In this field, count all of the WBCs present. Thus the total WBCs counted may be slightly more than 200.

5. If 10 or more parasites were found then calculate the results as follows:
   (No of parasites counted ÷ no of WBC counted) x 8000 (or patient's WBC count) = no of parasites/µl of blood

6. If after counting the 200th WBC the number of parasites is 9 or fewer, then continue counting until you reach at least 500 WBC and use the above formula to calculate the result.

7. Record the count of asexual forms of *P. falciparum* separately to the gametocyte count.

Limitations of method

Usually only *Plasmodium falciparum* parasites are quantified, not any of the other malaria species. This is because the non-falciparum species seldom reach densities of >1%, and the information is not clinically relevant.

Bibliography

1. Gilles HM & Warrell DA. (1993). Bruce-Chwatt's essential malariology, 3rd Ed. Edward Arnold, London.
2. Hydas Inc/NAMRU-2. 2005. Creation of validated blood smears for the microscopic diagnosis of malaria. Hydas Inc. Pennsylvania USA and NAMRU-2 Jakarta Indonesia. Supported by NIAID contract NO1-AI-85355 (MR4).
3. WHO. 1991. Basic malaria microscopy, Part 1 Learner's guide. WHO Geneva.
4. Ken Lilley. 2006. Australian Army Malaria Institute. Personal communication.

Procedure WHO 0017	***P. FALCIPARUM* ANTIGEN DETECTION USING THE ICT MALARIA P. F. CASSETTE TEST**		
Prepared by	**Date adopted**	**Signature**	
Leigh Dini & John Frean			

Revision date	**Revision signature**	**Review date**	**Review signature**

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EQA Microbiology Lab.	1		

Purpose

This SOP describes antigen detection of *Plasmodium falciparum* using the ICT malaria P.f. cassette test.

Principle

The test uses one antibody specific to the histidine-rich protein II (HRPII) antigen of *P. falciparum* that has been immobilized across the test strip.

Background and introduction

The ICT Malaria P.f. cassette test is a rapid, in vitro diagnostic test for the detection of circulating *P. falciparum* antigens. Reagent A, which is added to the blood in the cassette's well, lyses the whole blood and allows migration past the purple pad. The purple pad contains colloidal gold conjugated antibodies that are directed against HRPII antigen of *P. falciparum*. When a positive sample is applied, *P. falciparum* antigens bind to the gold conjugated antibodies. The antibody/antigen complexes continue migrating along the test strip where they are captured by immobilized antibodies. When capture occurs a pink control line will form in the window; when a negative sample is applied only the control line will appear. A procedural control line is also immobilized across the test strip and should always appear if the test has been performed correctly.

Responsibility

The medical technologist/scientist is responsible for performing the test and reporting of results.

Safety precautions

Reagent A contains sodium azide as a preservative. Sodium azide is toxic and should be handled carefully. Avoid ingestion or skin contact. Flush with a liberal amount of water when disposing of unwanted reagent.

Treat used cassettes as biohazard waste.

Universal precautions should be taken when dealing with blood specimens i.e. wear personal protective equipment; cover cuts and abrasions with adhesive dressings; dispose of sharps in a sharps container.

Frequency of testing

This test is performed on request.

Specimen type, collection and handling

Sample type: EDTA-treated (unclotted) blood or blood obtained by finger-prick.

Blood can be collected in an EDTA capillary tube after puncturing the patient's finger or heel, or by venipuncture into an EDTA tube. Universal precautions should be taken when dealing with blood.

- To obtain blood via puncture of a finger or heel: cleanse the skin with a sterile swab and dry with a sterile pad. Use a lancet to puncture the skin and collect the blood directly into the EDTA capillary tube. Perform the antigen test immediately.
- Collect venous blood by standard venipuncture procedure into an EDTA tube. If the test cannot be performed immediately, the blood may be stored for up to 3 days at 2–8 °C.

Equipment and materials

ICT malaria P.f. cassette test kit, Global Diagnostics (store at 15–30 °C; do not freeze)
Sterile alcohol swab
Lancet
Micropipette capable of delivering 10 µl and pipette tips
Timer

Procedure

1. Ensure that test components and blood samples are at room temperature prior to testing.

2. Remove cassette from the foil pouch.

3. Collect blood from a skin puncture in the capillary tube and ensure it is completely filled. Alternatively collect 10 µl of blood from an EDTA tube with a micropipette.

4. Slowly apply blood to the well.

5. Slowly add 5 drops of Reagent A to the well and start timer.

6. Read the results through the viewing window at 15 min. Strongly positive results may be visible sooner.

Interpretation of test results

Results read after 15 min may be inaccurate and should not be reported.
Control line: a control line (C) will form in the window if the test was performed correctly. The line appears blue before the test is run and changes to pink if the test was performed correctly.
Negative: a test is negative for *P. falciparum* if only the control line appears in the window.
Positive: a test is positive for *P. falciparum* if two lines appear in the window. As long as a control line appears, any visible test line (T) is a positive result, even if it is faint.
Invalid: a test is invalid if the control line (C) does not appear in the window, whether or not a test line (T) is present. The test should be repeated using a new cassette.

Limitations of method

Diagnosis should be made using the results of this test in conjunction with smear microscopy and clinical findings. This test only detects antigens from *P. falciparum*. Residual HRPII antigen may be detected several days following elimination of the parasite from the blood. This test is suitable for whole unclotted blood, not serum samples.

Internal quality control and external quality assessment procedures

A procedural control line is immobilized across the test strip and should always appear if the test has been performed correctly. Do not use kits past their expiry date. Do not mix reagents from different kits.

References

Package insert of ICT Malaria P.f. cassette test, Portland, USA (2003).

ANNEX 5

Examples of instructions and report forms sent to participants

5.1 Instructions (example of Survey September 2005)

5.2 Questionnaire

5.3 EQA report forms (bacteriology, malaria microscopy and mycobacteriology microscopy) (example of Survey September 2005)

5.4 EQA Global commentary (bacteriology, malaria microscopy and mycobacteriology microscopy) (example of Survey September 2005)

5.1 Instructions (example of Survey September 2005)

WHO / NICD

EXTERNAL QUALITY ASSESSMENT PROGRAMME
SEPTEMBER 2005

COUNTRY: **CONTACT PERSON:**

LABORATORY CODE:

ADDRESS:

TEL NO: **FAX NO:**

E-MAIL ADDRESS:

DATE RECEIVED IN LABORATORY:

DATE RETURNED TO NHLS:

RETURN THE RESULTS TO: Ms Vivian Fensham

STREET ADDRESS:	**POSTAL ADDRESS:**
WHO/ NHLS EQA Programme	WHO/ NHLS EQA Programme
NICD/NHLS	NICD/NHLS
North Block NU10	Private Bag X4
1 Modderfontein Road	Sandringham
Sandringham, Johannesburg	2131
South Africa	South Africa
TEL: +27 11 555 0344	**FAX: +27 11 555 0430**
E-MAIL: nhlswhoQA@nhls.ac.za	

RETURN DATE: 10 WORKING DAYS AFTER RECEIPT IN YOUR LABORATORY (TO REACH US NO LATER THAN THE 21st OCTOBER 2005)

If your laboratory is unable to complete the challenges contained in this survey, please return (by fax) just this page of the form, indicating which reason(s) most adequately explain the problem.

- ☐ **Laboratory reagents not available**
- ☐ **Laboratory equipment not functioning**
- ☐ **Laboratory staff on leave**
- ☐ **Survey contents not received in acceptable condition**
- ☐ **Other (Please state)**_____

- IF THE KIT CONTAINS BROKEN SPECIMENS, AUTOCLAVE THE CONTENTS AND DISCARD WITH MINIMAL EXPOSURE TO THE ATMOSPHERE. GUARD AGAINST THE PRODUCTION OF AEROSOLS, AS THESE CAN BE A SOURCE OF PERSONNEL AND LABORATORY CONTAMINATION.
- PLEASE TREAT THE SPECIMENS AS YOU WOULD NORMAL CLINICAL SPECIMENS.
- IT IS RECOMMENDED THAT YOU PHOTOCOPY ALL PAGES AND KEEP THESE FOR YOUR RECORDS.
- RETURN ALL ORIGINAL PAGES TO THE ABOVE ADDRESS BY E-MAIL, FAX, OR COURIER. IF YOU NEED TO COURIER DOCUMENTS TO US, (CONTACT V. FENSHAM AT +27 11 555-0344 FOR ACCOUNT DETAILS) OR POST.

Your laboratory has been enrolled in the following EQA programmes									
Enteric Pathogens	√	Meningitis Pathogens	√	Plague	√	TB microscopy	√	Malaria microscopy	√

Contact information

PLEASE CONTACT US IF YOU HAVE ANY PROBLEMS OR QUERIES

WHO/NICD EQA Programme Coordinators:

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5.2 Questionnaire

Indicate the best way to communicate with you:	**Telephone** ☐ **Fax** ☐ **E-mail** ☐
Global comments, feedback, positive and negative points and eventual suggestions to improve the programme:	

5.3 Report forms (example of survey September 2005)

BACTERIOLOGY IDENTIFICATION REPORT FORM

SAMPLE	SOURCE	CLINICAL DETAILS	INSTRUCTIONS
2005-3A Stool in Cary–Blair medium	**STOOL**	Bloody diarrhoea in a 30-year old male	Identify the pathogen/s and perform antimicrobial susceptibility testing.

2005-3A	
Details of primary media used for isolation:	
Colonial morphology (colony appearance on media):	
Biochemical reagents or tests used for organism identification: e.g. API, indole, etc. Please list the key reactions that you elicited in the identification process.	
Discuss further processing of this isolate e.g. serotyping:	
Final identification (i.e. report given to clinician):	

SUSCEPTIBILITY TESTING REPORT FORM

(NB This report format is used for survey numbers 2005-3A, 2005-3B, 2005-3C and 2005-3D)

Please report only those antimicrobials appropriate for the pathogen isolated and which are routinely tested. Please indicate carefully which antibiotics would be reported to the clinician.
Duplicate the form if more copies are required.

Survey number: 2005-3A
Isolate identification:
Method used for disc diffusion (Kirby–Bauer, Stokes' etc.):
Method used for MIC (broth MIC, agar MIC or E-test):
Media used:
Manufacturer of media:
Manufacturer of discs and size of discs (mm):
Source of breakpoint guidelines used (CLSI/NCCLS†, BSAC ‡, CA-SFM* or other): **†Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards** **‡ British Society for Antimicrobial Chemotherapy** ***Comité de l'Antibiogramme de la Société Française de Microbiologie**

ANTIMICROBIAL AGENT	Reported to clinician (Yes or No)	DISK CONCENTRATION (µg)	ZONE SIZE (mm)	MIC	[1]S	[2]I	[3]R

β-lactamase tested:	Method used:	Results:

[1]S= susceptible; [2]I = intermediate; [3]R = resistant

BACTERIOLOGY IDENTIFICATION REPORT FORM

SAMPLE	SOURCE	CLINICAL DETAILS	INSTRUCTIONS
2005-3B Stool in Cary-Blair medium.	**STOOL**	A patient with diarrhoea and severe dehydration in a refugee camp	Identify the pathogen/s and perform antimicrobial susceptibility testing.

2005-3B	
Details of primary media used for isolation:	
Colonial morphology (colony appearance on media):	
Biochemical reagents or tests used for organism identification: e.g. API, indole, etc. Please list the key reactions that you elicited in the identification process.	
Discuss further processing of this isolate e.g. serotyping:	
Final identification (i.e. report given to clinician):	

BACTERIOLOGY IDENTIFICATION REPORT FORM

SAMPLE	SOURCE	CLINICAL DETAILS	INSTRUCTIONS
2005-3C Glass slide with fixed unstained specimen/smear; Trans-isolate (TI) medium bottle with specimen. (Additional lyophilized specimen)	**CSF**	Fever and neck stiffness in an 8-month old infant.	Stain the smear appropriately and comment on your findings; process the TI medium appropriately and comment on your findings. **If there is no growth from your TI bottle, record this on this sheet. Then attempt to culture from the lyophilised vial. See attached sheet for instructions.** Perform all tests that are routine in your laboratory**.**

2005-3C	
Microscopic findings and preliminary report given to doctor:	
Details of processing of CSF:	
Final report given to doctor:	
Additional test results:	

BACTERIOLOGY IDENTIFICATION REPORT FORM

SAMPLE	SOURCE	CLINICAL DETAILS	INSTRUCTIONS
2005-3D Glass slide with fixed unstained specimen/smear; Trans-isolate (TI) medium bottle with specimen	**CSF**	An unconscious, pyrexial 9-year old child	Stain the smear appropriately and comment on your findings; process the TI medium appropriately and comment on your findings. Perform all tests that are routine in your laboratory

2005-3D	
Microscopic findings and preliminary report given to doctor:	
Details of processing of CSF:	
Final report given to doctor:	
Additional test results:	

BACTERIOLOGY IDENTIFICATION REPORT FORM

SAMPLE	SOURCE	CLINICAL DETAILS	INSTRUCTIONS
2005-3E Pus	**Bubo**	Fever and swelling in the groin in a male adult	Test for F1 antigen

2005-3E	
Details of test done:	
Test results:	
Your report to the doctor:	

SAMPLE	SOURCE	CLINICAL DETAILS	INSTRUCTIONS
2005-3F Pus	**Bubo**	A traveller, recently returned from India, developed a fever.	Test for F1 antigen

2005-3F	
Details of test done:	
Test results:	
Your report to the doctor:	

Malaria microscopy EQA

CLINICAL DETAILS AND INSTRUCTIONS FOR SAMPLES

SAMPLE	SOURCE	INSTRUCTIONS
2005-3-M1 Giemsa-stained thick & thin blood smears	**Human Blood**	Identify any parasites present and quantify the parasite load using your own procedure
2005-3-M2 Giemsa-stained thick & thin blood smears	**Human blood**	Identify any parasites present and quantify the parasite load using your own procedure
2005-3-M3 Giemsa-stained thick blood smears	**Human blood**	Identify any parasites present and quantify the parasite load using your own procedure
2005-3-M4 Giemsa-stained thick & thin blood smears	**Human blood**	Identify any parasites present and quantify the parasite load using your own procedure
2005-3-M5 Giemsa-stained thick & thin blood smears	**Human blood**	Identify any parasites present and quantify the parasite load using your own procedure
2005-3-M6 Giemsa-stained thick & thin blood smears	**Human blood**	Identify any parasites present and quantify the parasite load using your own procedure

Malaria identification report form

SAMPLE	ANSWER CODE (please refer to the list below)	PARASITE COUNT
2005-3-M1 Giemsa-stained thick & thin blood smears		
2005-3-M2 Giemsa-stained thick & thin blood smears		
2005-3-M3 Giemsa-stained thick blood smears		
2005-3-M4 Giemsa-stained thick & thin blood smears		
2005-3-M5 Giemsa-stained thick & thin blood smears		
2005-3-M6 Giemsa-stained thick & thin blood smears		

Please describe your procedure to quantify parasite load:

ANSWER CODES

CODE:	ANSWER:	CODE:	ANSWER:
P1	No parasite(s) seen	P5	*Plasmodium vivax*
P2	*Plasmodium falciparum*	P6	*Plasmodium* species
P3	*Plasmodium malariae*	P7	*Plasmodium* species, not *P. falciparum*
P4	*Plasmodium ovale*	P8	Relapsing malaria species (*P. ovale / P. vivax)*

Acid Fast Bacilli microscopy EQA (September 2005)

Instruction sheet for participating laboratories

Slides 2005-3-(T1-T4): Slides 1–4 have been made using concentrated sputum and stained by the EQA laboratory using the Ziehl-Neelsen method. Report on the presence of acid-fast bacilli and if present, supply a quantitative result. Complete the response sheet provided

Slides 2005-3-(T5-T7): Slides 5–7 have been made using concentrated sputum and fixed. Stain the slides using your laboratory's routine procedure. Record the staining procedure used. Report on the presence of acid-fast bacilli and if present, supply a quantitative result. Complete the response sheet provided

For all slides: Use the slide quantification scheme recommended by the International Union Against Tuberculosis and Lung Disease (reproduced below).
Different microscopists should complete separate response sheets. More than one response sheet may be returned per laboratory.

On completion: **Please fax / e-mail / post the completed response sheet to the appropriate laboratory, before the closing date.**

IUALTD Quantification Scheme for AFB Microscopy	
Carbol-fuchsin stain under oil (1000x)*	**Report**
No AFB	No acid-fast bacilli observed
1 to 9 AFB/100 fields	record exact figure
10 to 99 AFB/100 fields	1+
1 to 10 AFB/field	2+
>10 AFB/field	3+

*** If using fluorochrome staining, use appropriate conversion factors. See http://www.sahealthinfo.org/tb/tbmicroscopy.htm**

Mycobacteriology microscopy report form

Laboratory_____ Lab code no: _____ Survey no: 2005-3-slides (T1-T7)_

Date received in your laboratory: _____ Date results returned to EQA Unit: _____

Name of technician/technologist reading test smears: _____

Approximately how many specimens for TB microscopy does your laboratory receive per year?
_____

Approximately what proportions of specimens for TB microscopy are positive in your laboratory?
_____

SMEAR	TYPE of STAIN	RESULT
T1	Ziehl-Neelsen	
T2	Ziehl-Neelsen	
T3	Ziehl-Neelsen	
T4	Ziehl-Neelsen	
T5		
T6		
T7		

5.4 Global commentary (example of survey September 2005)

Overview of laboratory performance for Bacteriology Survey 2005-3
This survey comprised of 6 specimens (specimens A-F); two in each of the enteric, meningitis and plague disciplines. Also included in this survey for the first time were, 7 slides for tuberculosis microscopy (T1-7) and 6 thick and 5 thin slides for malaria microscopy (M1-6). Commentaries for these are published on separate pages. The survey was sent to a total of 72 laboratories of whom 16 received specimens in the plague discipline. A total of 62 responses were received. Ten laboratories failed to respond to this survey. The mean turn-around time of the courier was 4 days, and the mean response time of participating laboratories (from receipt of shipment to return of results to NICD) was 22 days (range 7-54). Table 1 below details the numbers of laboratories that obtained each possible score for all specimens in the survey.

Commentary on Bacteriology - Survey 2005 3

Many laboratories are performing poorly in the antimicrobial susceptibility testing grading areas; to facilitate improved responses, the method of evaluation of laboratory responses is explained clearly in Appendix 1. Please refer to this Appendix so that your laboratory may obtain maximum benefit from your participation.

Table1. Numbers of laboratories and scores obtained for specimens A-F, survey 2005 3.

Specimen	Grading area	Respondents	Number of laboratories					
F	F1 antigen	**62/72**	**4**	**3**	**1**	**0**	**NE**	**NG**
Simulated pus for F1 antigen	Culture and identification	62/72	54	0	0	3	5	0
A	Serotyping		15	18	5	19	5	0
Simulated stool with *Shigella dysenteriae* type 2	Antimicrobial selection		47	3	0	6	6	0
	Antimicrobial susceptibility results		40	6	2	8	6	0
B	Culture and ID	62/72	28	0	0	13	5	16
Simulated stool with *Vibrio cholerae* serogroup O1 serotype	Serotyping		17	5	1	18	5	16
	Antimicrobial selection		27	0	0	13	6	16
	Antimicrobial susceptibility results		24	3	0	13	6	16
C	Microscopy	62/72	35	0	0	23	4	0
Simulated CSF slide and TI medium with *Haemophilus influenzae* serotype c	Culture and ID		35	0	0	12	4	11
	Serotyping		1	6	0	40	4	11
	Antimicrobial selection		27	4	1	15	4	11
	Antimicrobial susceptibility results		3	1	0	43	4	11
D	Microscopy	62/72	50	0	0	8	4	0
Simulated CSF slide and TI medium with *Neisseria meningitidis* serogroup C	Culture and ID		52	0	0	5	4	1
	Serotyping		33	2	0	22	4	1
	Antimicrobial selection		33	15	1	7	5	1
	Antimicrobial susceptibility results		8	0	0	47	6	1
E	F1 antigen	13/16	7	0	0	4	2	
Simulated pus for F1 antigen								

NE=Not evaluated. Three laboratories were unable to complete surveys because their laboratory premises were relocating.

Specimen A

This specimen was a simulated stool inoculated with *Shigella dysenteriae* type 2.

Quality control of specimen A

The organism was present in the simulated stool specimen six weeks after preparation. All referee laboratories (Enteric Reference Laboratory, Centers for Disease Control and Prevention, USA; NHLS microbiology laboratories at Johannesburg General Hospital and Groote Schuur Hospital, Cape Town, South Africa and the Service de Biologie Clinique de l'Hôpital d'Instruction des Armées, Marseille, France) isolated a Shigella species and those referee laboratories with Shigella serotyping capacity typed the organism as 'type 2'.

Correct response

Culture and identification:	*Shigella* species
Serotyping:	*Shigella dysenteriae* type 2
Antimicrobials selected:	Nalidixic acid, trimethoprim-sulphamethoxazole, ampicillin, ciprofloxacin, chloramphenicol
Antimicrobial susceptibility reporting:	Sensitive to all above antibiotics

Issues highlighted by this EQA exercise

Culture and Identification; Antimicrobials selected; Antimicrobial susceptibility reporting
Culture and Identification was VERY well done with only 2 of 57 laboratories that provided identification results misidentifying the organism (*E. coli*, *Salmonella* species) and one laboratory failing to grow a pathogen. Antimicrobial choice and susceptibility testing results were also done well with more than 2/3 of laboratories obtaining acceptable results; however these grading areas were marked leniently without consideration of the methodology used; from 2006 antimicrobial susceptibility testing methodology will be evaluated. Please refer to Appendix 1 to ensure that your laboratory completes the Susceptibility Testing Report Form correctly.

Serotyping:
Shigella dysenteriae type 1 (Sd1) is responsible for epidemic dysentery and haemolytic uraemic syndrome because of the presence of the toxin gene encoded for by *stx1*. The other *S. dysenteriae* types (types 2-15) do not contain this toxin gene and produce milder disease; Sd2 is the most common serotype after Sd1 and may cause small-scale outbreaks. A large outbreak of dysentery caused by Sd1 originated in East Africa in the 1960s and spread to Southern Africa by the 1980s. It is essential that National Public Health laboratories (NPHLs) be able to differentiate between type 1 and non-type 1 strains because of the major public health impact this organism can have. It is acceptable for laboratories that do not have typing sera to refer isolates on to NPHLs for typing. Three laboratories indicated that the organism was *S. dysenteriae* type 1. Regarding serotyping, scores were allocated as follows:

Serotyping	**4**	*Shigella dysenteriae* (type 2) or *Shigella dysenteriae* not type 1
	3	*Shigella dysenteriae* serotype not stated
	3	Referred for typing
	1	*Shigella dysenteriae* type 1
	0	Not typed / typing sera not available
	0	Incorrect ID / No pathogens isolated / Not done
	NG	No growth

Specimen B

The specimen was a simulated stool inoculated with an ATCC isolate of *Vibrio cholerae* serogroup O1 serotype Ogawa.

Quality control of specimen B

At the NICD, quality control of 10% of the shipment indicated that the simulated stool specimen contained viable *Vibrio cholerae* serogroup O1, serotype Ogawa until 34 days after preparation. The Enteric Reference Laboratory of the Centers for Disease Control, Atlanta, USA; and the NHLS Microbiology laboratories at the Johannesburg General Hospital and Groote Schuur Hospitals, South Africa confirmed the presence and identification of this organism using biochemistry, serology and molecular tests. One referee lab (the Service de Biologie Clinique de l'Hôpital d'Instruction des Armées, Marseille, France) found no growth in the sample; therefore laboratories that failed to obtain growth of this pathogen were not penalised but rather recorded as 'No growth obtained'.

Correct response

Culture and identification:	*Vibrio cholerae*
Serotyping:	*Vibrio cholerae* serogroup O1 serotype Ogawa
Antimicrobials selected:	Nalidixic acid, trimethoprim-sulphamethoxazole, furazolidone, ciprofloxacin, tetracycline
Antimicrobial susceptibility reporting:	Sensitive to all of the above antibiotics

Issues highlighted by this EQA exercise

Culture and Identification:
Sixteen of 62 laboratories (26%) failed to obtain growth. In subsequent surveys the NICD will try to address this issue to ensure that laboratories have maximum opportunity to culture the pathogen. Misidentifications included *Acinetobacter, Aeromonas, Pasteurella, Plesiomonas* (1 laboratory each), *Shigella* species (5 laboratories) and four laboratories that identified and reported staphylococci.

Serotyping:
Only *Vibrio cholerae* serogroups O1 and O139 have epidemic potential; therefore any report of *Vibrio* species should indicate the serogroup of the isolate. Laboratories failing to mention the serogroup, but indicating the correct serotype obtained 3 points. Two laboratories (both from the same country!) indicated that the specimen was a non-O1, non-O139 serogroup. One laboratory named the organism a serotype Inaba. These three laboratories should ensure that their antisera are controlled on organisms of known serogroup and serotype. Laboratories not having antisera obtained 0.

Antimicrobial susceptibility testing and results:

Antimicrobial choice and susceptibility testing results were done well; more than 90% of laboratories that grew the organism obtained acceptable results; however these grading areas were marked leniently without consideration of the methodology used; from 2006 antimicrobial susceptibility testing methodology will be evaluated. Please refer to Appendix 1 to ensure that your laboratory completes the Susceptibility Testing Report Form correctly.

Specimen C

Laboratories received a lyophilized sample inoculated with an ATCC strain of *Haemophilus influenzae* serotype c and an unstained smear containing Gram-negative coccobacilli and polymorphonucleocytes.

Quality control of specimen C

All reference laboratories confirmed the presence of Gram-negative cocco-bacilli on the slides. *Haemophilus influenzae* was found to be present in the lyophilised specimen for 83 days after specimen preparation. The NHLS microbiology laboratories at the Johannesburg General Hospital and Groote Schuur Hospital (Cape Town) obtained growth of the *Haemophilus influenzae* and referred the isolate for serotyping. The Meningitis and Special Pathogens Reference Laboratory of the Centers for Disease Control obtained growth of a *Haemophilus influenzae* serotype c and Unite du meningocoque WHO collaborating Center of the 'Service de Santé des Armées' named the organism '*Haemophilus influenzae* not type b'.

Correct response: specimen C

Microscopy	Gram-negative cocco-bacilli / bacilli
Culture and ID	*Haemophilus influenzae*
Serotyping	*Haemophilus influenzae* serotype c
Antimicrobial choice	Ampicillin, amoxicillin/clavulanate, ceftriaxone, chloramphenicol
Antimicrobial results	Guidelines stated, correct methodology, correct results (sensitive to all tested), β-lactamase negative.

Issues highlighted by this EQA exercise

Microscopy:

Fourteen laboratories reported 'No organisms seen' on the simulated CSF slide included in the shipment. These laboratories should review the slides; the organisms are present! Six laboratories saw Gram-negative cocci. This response was penalised as it may mislead clinicians to consider *Neisseria meningitidis* as the causative organism with consequent treatment implications. Two laboratories reported Gram-positive organisms. Quality control of Gram's stain with known Gram-positive and Gram-negative organisms is essential.

Culture and ID:

All 35 laboratories that obtained correct microscopy results also correctly cultured and identified the organism as *Haemophilus influenzae*. This indicates that these laboratories carefully correlated their culture and Gram-stain results. Well done! Misidentifications included *Neisseria meningitidis* (4 laboratories), *E. coli* (2 laboratories), *Acinetobacter,*

Providencia, Staphylococcus and Streptococcus pneumoniae (1 laboratory each); each of these results were obtained by laboratories that also reported an incorrect microscopy result.

Serotyping:
This grading area was very poorly performed; 14 laboratories reported that the organism was serotype b. All these laboratories did not perform serotyping with specific antisera; rather they used the bacterial latex antigen detection kit. Both the Slidex and Pasteurex when tested in our laboratory with this organism failed to give a positive result with the *Haemophilus influenzae* type b antisera. Laboratories need to have confidence in their techniques; if the organism is identified as a *H. influenzae* using biochemical tests, but the bacterial latex agglutination test is negative and all controls have been performed, it is safe to report this organism as 'non-type b'. Countries that have experienced integration of the conjugate *Haemophilus influenzae* type b vaccine into their Expanded Programme on Immunisation against childhood diseases may see an increase in non-type b invasive isolates of *H. influenzae*; consequently they need to take special care to identify non-type b isolates. Appendix 2 below provides an outline for interpretation of serotyping reactions. Marks were allocated as follows:

Serotyping	**4**	*Haemophilus influenzae* serotype c
	3	Referred for typing / non-specific reactions, not able to type and referred / not type b
	3	Non-specific typing reaction; unable to type isolate, therefore isolate referred for typing
	3	*Haemophilus influenzae* not serotype b
	1	Non-typeable or agglutination with all monovalent serotyping reagents
	0	Incorrect serotype
	NG	No growth

Antimicrobial choice:
Appropriate antibiotics for treating meningitis caused by *Haemophilus influenzae* include ampicillin, cefotaxime/ceftriaxone and chloramphenicol. The carbapenems are also appropriate though expensive and not widely available. Laboratories were not penalised for testing additional or inappropriate antibiotics, as long as the choice included β-lactam agents. It was not acceptable to omit ampicillin and a third generation cephalosporin. Laboratories should refer to the appropriate antimicrobial susceptibility testing guidelines when choosing which antibiotics to test.

Antimicrobial results:
The guidelines in Appendix 1 below were followed when the NICD evaluated laboratory responses in this grading area; laboratories performed poorly as many did not state which guidelines they followed and even fewer followed their stated methodology correctly. The testing methodologies required by the different AST guidelines are listed below in Table 2.

Table 2. Disc diffusion testing methodologies[#] for *Haemophilus influenzae* according to Clinical Laboratory Standards Institute (CLSI), Société Française de Microbiologie (SFM) and British Society for Antimicrobial Agents and Chemotherapy (BSAC)

	CLSI	SFM	BSAC
Disc diffusion method:	Kirby-Bauer	Kirby-Bauer	Kirby-Bauer
Media	Haemophilus test media	Chocolate agar with PolyViteX® Or Haemophilus test media	IsoSensitest Agar + 5 % defibrinated horse blood + 20 mg/L NAD
Incubation conditions:	35 °C ±2 degrees, 5% CO2;	35-37° C, atmosphere not specified	35-37°C in 4-6% CO2
Duration of incubation	16-18 hours	18-24 hours	18-20 hours
Antibiotic content			
Ampicillin	10µg	2µg	2µg
Chloramphenicol	30µg	30µg	10µg
Cefotaxime/ceftriaxone	30µg	*	30µg

[#]Consult the respective guidelines for interpretive zone diameters, as the guidelines differ.

*Interpretive zone diameters for disc testing not provided, therefore this agent cannot be tested using these guidelines.

Specimen D

Laboratories received an unstained slide prepared from simulated CSF, a Trans-Isolate medium inoculated with an ATCC strain of a *Neisseria meningitidis* serogroup C. The slide contained polymorphonucleocytes and numerous Gram-negative diplococci.

Quality control of specimen D

All referee laboratories observed Gram-negative diplococci on the smear provided with the shipment. *Neisseria meningitidis* was found to be present in the Trans-Isolate medium for 41 days after specimen preparation. The TI bottles showed no contamination. The Meningitis and Special Pathogens Reference Laboratory of the Centers for Disease Control and the Unite du meningocoque WHO collaborating Center of the 'Service de Santé des Armées' in Marseille confirmed the presence and identification of this organism using microscopy and biochemical and serological tests. The NHLS microbiology laboratories at the Johannesburg General Hospital and at Groote Schuur Hospital, Cape Town identified the organism as a *Neisseria meningitidis* and referred it to the RMPRU Unit at the NICD for serogrouping. The three referee laboratories that performed susceptibility testing confirmed that the isolate was susceptible to all antibiotics.

Correct response: Specimen D

Microscopy	Gram-negative diplococci/ cocci and polymorphonuclear leucocytes
Culture and ID	*Neisseria meningitidis*
Serotyping	*Neisseria meningitidis* serogroup C
Antimicrobial choice	Penicillin/ampicillin, +/-ceftriaxone/cefotaxime, chloramphenicol, rifampicin, ciprofloxacin
Antimicrobial results	Guidelines stated, correct methodology, sensitive to all tested

Issues highlighted by this EQA exercise

Microscopy:
In total, 50 laboratories obtained the correct microscopy result; however 6 laboratories did not see organisms on the slide, and three laboratories reported 'Gram-positive cocci' present.

Culture and Identification:
Of the 52 laboratories that obtained the correct culture result, 4 reported no organisms seen on the Gram's stain, and one laboratory reported 'Gram-positive diplococci'. It is important that laboratories review both their culture and microscopy results when these are discordant. Often organisms can be found on careful review of the slide, or it can be concluded that the Gram's stain was under- or over-decolourised resulting in a microscopy error. Misidentifications included *Alkaligenes*, *Streptococcus pneumoniae* and *Streptococcus* species (one laboratory each). Three laboratories failed to grow the pathogen.

Serotyping:
Thirty-eight laboratories performed serogrouping of which 33 reported that the organism was a serogroup C. Two laboratories reported that the organism was within serogroups A-D, and this was regarded as acceptable but awarded 3 points. Three laboratories reported incorrect serogrouping results (A, W135, Y/W135). Two of these countries are within the meningitis belt of Africa.

Antimicrobial choice:
Antibiotics appropriate for the treatment of meningococcal meningitis include penicillin (to which almost all meningococci remain susceptible), 3rd generation cephalosporins and chloramphenicol. Three laboratories omitted to test penicillin or ampicillin but tested a 3rd generation cephalosporin and were awarded 3 point. Chemoprophylaxis (ceftriaxone, ciprofloxacin or rifampicin) can be given to close contacts of the diseased individuals in order to eradicate oropharyngeal carriage. Therefore these agents should also be tested for susceptibility. Twelve laboratories omitted to test any agent that can be used for chemoprophylaxis. Rifampicin resistance, although rare, does occur. Choice of antibiotics was graded as follows:

Antimicrobial choice	Grading	
	4	Penicillin/ampicillin, +/-ceftriaxone/cefotaxime, chloramphenicol, rifampicin, ciprofloxacin
	3	Omission of antibiotic used for secondary prophylaxis (rifampicin, ciprofloxacin, ceftriaxone)
	3	Omission of penicillin, but inclusion of ceftriaxone/cefotaxime, chloramphenical, rifampicin, ciprofloxacin
	1	Omission of any appropriate beta-lactam
	0	No appropriate antibiotics
	0	Incorrect ID/ not done/ No growth

Antimicrobial susceptibility testing results:
As with specimen C above, the guidelines in Appendix 1 below were followed when the NICD evaluated laboratory responses in this grading area; as with specimen C above, laboratories performed poorly as many did not state which guidelines they followed and even

fewer followed their stated methodology correctly. The testing methodologies required by the different AST guidelines are listed below in Table 3. Difficulty arises for laboratories that follow CLSI methodology because disc testing of meningococci is not recommended. Options available for these laboratories include:

- Adherence to SFM or BSAC guidelines for testing of meningococci
- Referral of isolates to a centre that is able to perform MIC testing methodology
- Purchasing of penicillin Etest strips only (penicillin testing is essential; chemoprophylaxis antibiotics could be omitted where cost factors are a consideration).

Only 8 laboratories adhered to their stated guidelines correctly; these laboratories used the following guidelines: SFM (5 laboratories), CLSI (3 laboratories).

Table 3. Disc diffusion testing methodologies[#] for *Neisseria meningitidis* according to Clinical Laboratory Standards Institute (CLSI), Société Française de Microbiologie (SFM) and British Society for Antimicrobial Agents and Chemotherapy (BSAC)

	SFM	**BSAC**	**CLSI**
Disc diffusion method:	Kirby-Bauer, recommended only for oxacillin, chloramphenicol and rifampicin	Kirby-Bauer	Disc testing is not recommended for meningococci
Media	Mueller-Hinton agar + 5% sheep blood	IsoSensitest Agar + 5 % defibrinated horse blood	
Incubation conditions:	35-37℃ in 5% CO2	35-37℃ in 4-6% CO2	
Duration of incubation	18-20 hours	18-20 hours	
Antibiotic content			
Penicillin	*	1 unit	
Ampicillin	*	*	
Oxacillin	5µg or 1µg	*	
Cefotaxime	*	5µg	
Rifampicin	30µg	2µg	
Chloramphenicol	30µg	10µg	
Ciprofloxacin	*	1µg	

[#]Consult the respective guidelines for interpretive zone diameters, as the guidelines differ.

*Interpretive zone diameters for disc susceptibility testing not provided, therefore this agent cannot be tested using these guidelines.

Specimen E and F

Laboratories received specimens E and F which were both simulated pus specimens from a bubo aspirate. Included in the package were F1 antigen detection dipsticks. Unfortunately instructions for performing the test were omitted and one laboratory had not seen or used the test before. This rapid dipstick test is manufactured by Pasteur Institute, Madagascar and is reported as having 100% specificity and 100% sensitivity.

Quality control of specimen E and F

The Special Bacterial Pathogens Unit of the NICD and the Pasteur Institute of Madagascar found a negative dipsticks result on both Specimens E and F. The Bacterial Zoonoses Branch of the CDC at Fort Collins, USA obtained a positive result for Specimen E and a negative result for specimen F. The NICD repeated these tests, and confirmed the negative results. On discussion with the CDC and on observation of the dipsticks on repeating, it became clear that if the tests are read AFTER 15 minutes, some tests give a slight faint band leading to false-positive results. Laboratories need to be sure to read the dipsticks within 15 minutes of preparation.

Correct response: Specimen E and F

Specimen E	**F1 antigen**	Negative
Specimen F	**F1 antigen**	Negative

Issues highlighted by this EQA exercise

The laboratory that did not have the test method was not evaluated. False positive results were obtained for Specimens E and F by 2 and 4 laboratories respectively. Adherence to the testing protocol and reading results within 15 minutes of performing the test are essential to avoid false positive results.

Appendix 1, Bacteriology Commentary: Evaluation of laboratory responses.

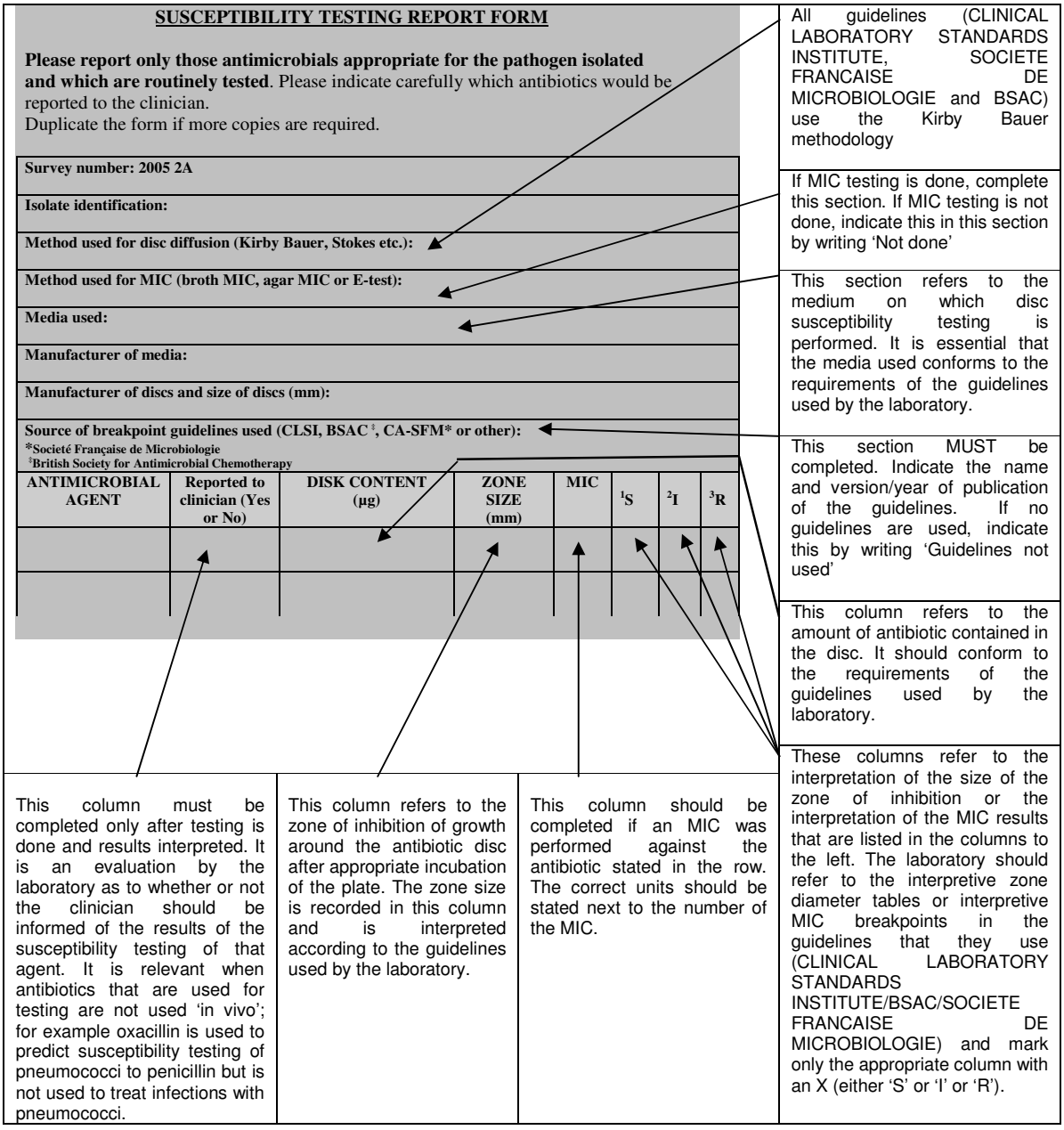
Laboratory responses are graded as 'Acceptable', (allocated a score of 4 or 3) or 'Unacceptable' (allocated a score of 1 or 0). An 'Acceptable' response is one that is microbiologically correct, and which will lead to a correct response in terms of clinical case and public health management. Minor microbiological errors are assigned a score of '3'. An 'Unacceptable' response is one that is microbiologically incorrect, and will not lead to a correct response in terms of public health management. If aspects of the response are correct, but the entire response is incorrect, a score of 1 will be assigned. In each grading area the NICD Technical Implementation Group decides which responses are acceptable, and defines these in an objective way. The commentary gives an explanation for the allocated scores.

With regard to the 'Antimicrobial susceptibility reporting' grading area, it is essential that laboratories complete the response form correctly and do not omit any information. If the NICD response form is not used, the laboratory must be sure to submit all the information required in the response form to the NICD. The NICD evaluates laboratory responses according to the antimicrobial susceptibility guidelines used; laboratories must follow the methodology stated in the appropriate guidelines (CLSI, SFM or BSAC). Errors in methodology mean that antimicrobial susceptibility testing results cannot be interpreted; in fact, failure to conform to a published antimicrobial susceptibility testing methodology means that antimicrobial susceptibility testing is meaningless!! The NICD cannot evaluate antimicrobial susceptibility testing results fairly or objectively when laboratories do not state which guidelines they follow. Refer to Figure 1 below which highlights important sections of the response form that must be completed. Table 1 shows as an example, how scores were assigned for Specimen 3C of Survey 2005 3.

Table 1. Possible responses and assignment of scores for Specimen 3C of Survey 2005 3.

Microscopy	**4**	Gram-negative cocco-bacilli / bacilli
	0	No bacteria seen or incorrect gram stain
Culture and ID	**4**	*Haemophilus influenzae*
	0	Incorrect ID
	NG	No growth
	C	Contaminated
Serotyping	**4**	*Haemophilus influenzae* serotype c
	3	Referred for typing /non-specific reactions, not able to type and referred/ Not type b
	1	Non-typeable or agglutination with all monovalent serotyping reagents
	0	Incorrect serotype
	NG	No growth
	C	Contaminated
Antimicrobial choice	**4**	Ampicillin, amoxicillin/clavulanate, ceftriaxone, chloramphenicol
	3	Testing amoxicillin instead of ampicillin (but also tested a 3rd generation cephalosporin)
	1	Testing inappropriate β-lactams antibiotics
	0	Incorrect ID or not done
	NG	No growth
	C	Contaminated
Antimicrobial results	**4**	Guidelines stated, correct methodology, correct results,
	3	Guidelines stated, correct methodology, minor error in results
	0	Guidelines not stated or incorrect methodology (regardless of results obtained)
	0	Incorrect ID or not done
	NG	No growth
	C	Contaminated

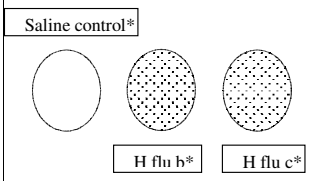
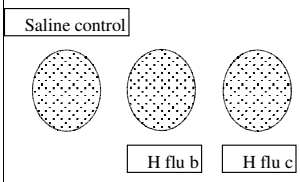
Figure 1. Completion of Susceptibility Testing Report Form

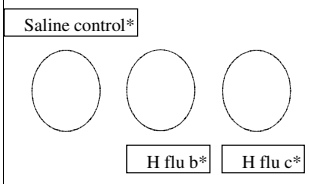
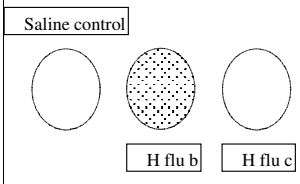


Appendix 2: Reporting of serotyping/serogrouping results for meningitis pathogens

Guide to reporting serotyping/grouping results for meningitis pathogens

The identification of bacteria must be confirmed before commencing with serotyping/serogrouping.
This guide assumes all antisera and reagents are quality controlled, and positive and negative controls are working as stipulated.
*Examples are given with saline control and antisera for *Haemophilus influenzae* serotype b and c, however the guide is also relevant to normal saline with antisera for *Neisseria meningitidis* serogroup A, B, C etc. or antisera for other *H. influenzae* serotypes.

Term	Laboratory criteria	Action	Report to clinician/public health expert	
Non-specific agglutination/cross reaction	Agglutinates in more than one of specific antisera	-Subculture and try again from a younger overnight culture -Subculture overnight in a serum-enriched broth, then centrifuge and use sediment for typing. A fresh subculture of the broth on blood or chocolate agar or 10% blood agar, sometimes produces a 'more mucoid' strain enhancing capsule production	**Non-typeable/Non-groupable** due to **non-specific agglutination**	
Auto-agglutination/rough	Agglutinates in normal saline	-Subculture and try again from a younger overnight culture -Subculture overnight in a serum-enriched broth, then centrifuge and use sediment for typing. A fresh subculture of the broth on blood or chocolate agar or 10% blood agar, sometimes produces a 'more mucoid' strain enhancing capsule production	**Non-typeable/Non-groupable** due to **auto-agglutination**	

Non-reactive	No agglutination with specific anti-sera or with the saline control	No action if all controls have worked.	**Non-typeable/non-groupable**; if not all antisera are tested then only those that are negative should be reported (*e.g. Haemophilus influenzae* not serotype b or c; or *Neisseria meningitidis* not serogroup A,B,C)	
Specifc agglutination	Rapid agglutination by a specific antiserum only, (bacterial cells have clumped and background appears clear), and the absence of agglutination in the saline control	If polyvalent positive, repeat with individual sera.	*Haemophilus influenzae* serotype b	

For *Haemophilus influenzae* antisera, laboratories may want to test against all antisera to ensure greater accuracy.
Above adapted in part from Perilla M, Ajello G, Bopp C *et al*. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World, World Health Organization, 2003.

(1) Bokermann S, Zanella RC, Lemos AP, de Andrade AL, Brandileone MC. Evaluation of methodology for serotyping invasive and nasopharyngeal isolates of *Haemophilus influenzae* in the ongoing surveillance in Brazil. J Clin Microbiol 2003 Dec;41(12):5546-50.

(2) LaClaire LL, Tondella ML, Beall DS, Noble CA, Raghunathan PL, Rosenstein NE, et al. Identification of *Haemophilus influenzae* serotypes by standard slide agglutination serotyping and PCR-based capsule typing. J Clin Microbiol 2003 Jan;41(1):393-6.

Commentary on Malaria Microscopy Survey 2005-3

General information

Please only report your final diagnosis based on thick and thin smear microscopy for each challenge. However, it may not always be possible for us to provide both a thick and thin smear for each challenge. The rationale for the scoring system is explained in Table 1 and the scoring system for the current survey is explained in Table 2. The parasite counts were not assessed for this survey, but will be assessed in future surveys.

Table 1: Rationale for scoring system

Score:	Result:	Definition:	Performance assessment:
4	Completely correct result	A result accepted as the most correct and clinically relevant result.	Acceptable
3	Almost completely correct result	A result not entirely correct but having little or no clinical impact; a deviation from what is considered the most clinically relevant result.	Acceptable
	Separator	*To divide the acceptable from unacceptable responses.*	N/A
1	A significantly incorrect result	A clinically relevant result that could lead to a diagnosis or treatment error.	Unacceptable
0	Completely incorrect result	A clinically relevant result that could lead to a **major** diagnosis or treatment error.	Unacceptable
0	No result	No result submitted by participant.	Unacceptable

Table 2: Scoring system for current survey (Codes P1-P8 explained below*)

Challenge no	Description of challenge	Expected result (code)	Expected result (text)	4	3	1	0
M1	Thick and thin smear	Not applicable	Not assessed				
M2	Thick and thin smear	P1	No parasite/s seen	P1	N/A	N/A	Any other response
M3	Thick smear only	P2	*Plasmodium falciparum*	P2	P6	P2 + another malaria species	Any other response
M4	Thick and thin smear	P2	*Plasmodium falciparum*	P2	P6	P2 + another malaria species	Any other response
M5	Thick and thin smear	P2	*Plasmodium falciparum*	P2	P6	P2 + another malaria species	Any other response
M6	Thick and thin smear	P1	No parasite/s seen	P1	N/A	N/A	Any other response

*P1 No parasite/s seen, P2 *Plasmodium falciparum*, P3 *Plasmodium malariae*, P4 *Plasmodium ovale*, P5 *Plasmodium vivax*, P6 *Plasmodium species*, P7 *Plasmodium* species not *P.falciparum*, P8 Relapsing malaria species (*P.ovale/ P.vivax*).

Overview of laboratory performance for Malaria Microscopy Survey 2005-3

Table 3. Summary of laboratory responses for Specimens M1-6, Survey 2005-3

Slide	M1	M2	M3	M4	M5	M6
Expected response	Not evaluated	No parasites seen	*Plasmodium falciparum*	*Plasmodium falciparum*	*Plasmodium falciparum*	No parasite/s seen
Malaria microscopy not done in recipient laboratory	**2**	**2**	**2**	**2**	**2**	**2**
Laboratory unable to complete response sheet	**4**	**4**	**4**	**4**	**4**	**4**
Score 4	**0**	**41**	**33**	**30**	**40**	**41**
Score 3	**0**	**0**	**3**	**0**	**1**	**0**
Score 1	**0**	**0**	**1**	**1**	**1**	**0**
Score 0	**0**	**15**	**19**	**25**	**14**	**15**
Not evaluated	**56**	**0**	**0**	**0**	**0**	**0**
No return	**10**	**10**	**10**	**10**	**10**	**10**
TOTAL	**66**	**66**	**66**	**66**	**66**	**66**

Specimen M1: This challenge consisted of a thick and thin Giemsa-stained blood smear. There was no consensus amongst the referees and us, nor amongst the EQA participants, so this challenge was not assessed.

Specimen M2: This challenge consisted of a thick and thin Giemsa-stained blood smear containing no parasites. There was 100% consensus amongst the referees and 73% of participants who responded achieved an acceptable score (3 or 4).

Specimen M3: This challenge consisted of a Giemsa-stained thick blood smear containing only *Plasmodium falciparum* parasites. There was 80% consensus amongst the referees and one referee reported *Plasmodium* species present. It is better to report *Plasmodium* species present when one is unsure of the species diagnosis, as this will not mislead the doctor. 64% of participants who responded achieved an acceptable score (3 or 4).

Specimen M4: This challenge consisted of a thick and thin Giemsa-stained blood smear containing *Plasmodium falciparum* parasites. There was 100% consensus amongst the referees and 54% of participants who responded achieved an acceptable score (3 or 4).

Specimen M5: This challenge consisted of a thick and thin Giemsa-stained blood smear containing *Plasmodium falciparum* parasites. There was 100% consensus amongst the referees and 73% of participants who responded achieved an acceptable score (3 or 4).

Specimen M6: This challenge consisted of a thick and thin Giemsa-stained blood smear containing no parasites. There was 100% consensus amongst the referees and 73% of participants who responded achieved an acceptable score (3 or 4).

Commentary on Tuberculosis Microscopy Survey 2005 3

Background to the Tuberculosis Microscopy Survey

Tuberculosis smear microscopy is the cornerstone of diagnosis of pulmonary tuberculosis. It has the advantage of being a cheap and easily performed test. However in order to maximise the diagnostic opportunity afforded by this test, it is essential that it be quality controlled; to this end the WHO and Association of Public Health Laboratories and Centers for Disease Control, Atlanta USA published a document entitled 'External Quality Assessment for AFB Smear Microscopy' in 2002. Through a donation from the Netherlands government to WHO Office in Lyon, a TB smear microscopy EQA programme has been added onto the existing WHO/AFRO bacteriology EQA programme. This EQA programme follows the WHO guidelines in the publication above on proficiency testing or panel testing of TB microscopy. According to this scheme, errors in slide microscopy are classified as 'Quantitation error, High- or Low false positive or High or Low false negative (according to the table in the Standard Operating Procedure WHO 0012 in Appendix 4B in this document).

Overview of laboratory performance for Tuberculosis Microscopy Survey 2005-3

This survey comprised of 7 slides (Slide T1-7) of which T1-4 were stained and T5-7 were unstained. It was sent to all 72 laboratories that participate in the EQA programme. A total of 43 laboratories were able to participate in the survey and provide results. In total 576 slides were reviewed by participating laboratories; 7 major and 15 minor errors were made. Table 1 below details the numbers of laboratories that obtained each possible score for all specimens in the survey. Most problems were encountered with the unstained slides, where the variability in responses was greater than with the stained slides.

Table1. Numbers of laboratories and scores obtained for specimens A-F, survey 2005 3.

Slide	Expected response	Respondents	Number of laboratories			
			Correct	Quantitation Error	High False Positive/ Negative	Low false positive/ Negative
T1	AFB not observed	43	42	-	-	1
T2	2+	43	41	2	-	-
T3	2+	43	37	5	1	
T4	AFB not observed	43	43	-	-	-
T5	2+	41	31	6	4	-
T6	AFB not observed	42	41	-	-	1
T7	AFB not observed	42	40	-	2	-

ANNEX 6

Financial background of WHO/NICD EQA programme

8.1 Financial resources

There are no subscription fees for participation in the WHO/NICD EQA programme and all funding is provided by WHO and external voluntary contributions (either direct or in kind). In addition to resources provided through the WHO Office in Lyon and the WHO Regional Office for Africa, WHO gratefully acknowledges support from:

- Centers for Disease Control and Prevention, Atlanta, USA (in-kind support)
- GAVI through the WHO Regional Paediatric Bacterial Meningitis Unit (direct support)
- National Institute for Communicable Diseases, Johannesburg, Republic of South Africa (in-kind support)
- Referee laboratories (in-kind support)
- The Government of the Kingdom of the Netherlands (direct support, malaria and tuberculosis component)
- United States Agency for International Development.

8.2 Budget categories

The budget for Year 4 (2005–2006) of the WHO/NICD EQA programme was US$ 93 501. This sum was apportioned as shown in Table 1 below. In addition, NICD provided the salaries of professional staff in the Technical Implementation Group (see Annex 1), except the salaries mentioned below.

Table 1 Budget categories

Bacteriology EQA programme	**US $**
Personnel costs (technologist half-time), office supplies, reporting	26 336
Packaging and shipping	34 444
Translation	1 493
Laboratory reagents, consumables, hardware	2 948
Regional advisory group meeting 2006	3 008
Sub-total (bacteriology)	68 229
Microscopy EQA programmes (malaria and TB)	
Training for organizer	4 000
Junior medical technologist	9 552
Packaging and shipping	8 257
Laboratory reagents, consumables, hardware (malaria)	2 808
Laboratory reagents, consumables, hardware (TB)	655
Sub-total (malaria and TB)	25 272
TOTAL	**93 501**