Table of Contents

Acronyms

AST	Antibiotic Susceptibility Testing
AMR	Antimicrobial Resistance
ATCC	American Type Culture Collection
CQI	Continuous Quality Improvement
EIDL	Enteric and Invasive Disease Laboratory
EQAS	External Quality Assessment Scheme
IATA	International Air Transport Association
IQA	Internal Quality Assurance
NEQAS	National External Quality Assurance Scheme
NRL	National Reference Laboratory
PL	Participating Laboratory
PT	Proficiency Testing
QA	Quality Assurance
QC	Quality Control
RCDC	Royal Center for Disease Control
SIR	Susceptible, Intermediate, Resistant
SoP	Standard Operating Procedure
TQM	Total Quality Management
WHO	World Health Organization

List of Tables and figures

Foreword

The guideline prescribes detailed information on the importance and benefits of National External Quality Assessment Scheme (NEQAS) requirements in every microbiological laboratory. Also, to organize and conduct an External Quality Assurance System (EQAS) on susceptibility testing of enteric bacterial pathogens. Any proposed deviations from these guide specifications and design guidelines require approval from the Enteric and Invasive Disease Laboratory (EIDL) under the Royal Center for Disease Control (RCDC).

This guidance document is designed for all microbiological practicing laboratories required or aiming to establish and manage NEQAS for testing of microbiological identification parameters.

The purpose of this document is to support laboratories to assess and if necessary, improve the quality of bacterial identification and susceptibility testing of bacterial pathogens from clinical samples. Furthermore, to assess and improve the comparability of surveillance and antimicrobial susceptibility data reported by different laboratories. External Quality Assessment (EQA) is now required for Good Laboratory Practice within all laboratories as part of the quality assurance and quality improvement programs for diagnostic testing in general. In many countries, EQA is required for laboratory accreditation. EQA is one of the components of a total quality assurance program of all laboratories.

Acknowledgements

The Enteric and Invasive Disease Laboratory (EIDL) under the Royal Center for Disease Control (RCDC) would like to express our profound gratitude to Professor Benjamin Howden, Courtney Lane, Sarah Baines and Chantel Lin from Doherty Institute, Melbourne University, Victoria, Australia for their continuous guidance, from the commencement of development of this guideline till the end.

The work was supported by the UK Fleming Fund Project for Antimicrobial Resistance and we remain highly indebted to the project. We are grateful to the Technical Advisory Group for their commitment and continuous guidance throughout the development of this document.

Last, but not the least, we wish to express gratitude to the following authors for their extended editions, valuable contributions, and the hard work in developing this guideline.

1. Tshering Dorji, Deputy Chief Laboratory Officer, Royal Center for Disease Control, Thimphu
2. Kinley Gyem, Sr. Laboratory Officer, Royal Center for Disease Control, Thimphu
3. Sonam Pelden, Chief Laboratory Officer, Royal Center for Disease Control, Thimphu
4. Jit Bdr Darnal, Clinical Officer, Royal Center for Disease Control, Thimphu
5. Dorji Tshering, Sr. Laboratory Technician, Royal Center for Disease Control, Thimphu
6. Kinley Penjor, Sr. Laboratory Officer, Royal Center for Disease Control, Thimphu
7. Sangay Dorji, Laboratory Technician, Royal Center for Disease Control, Thimphu.
8. Purmila Gurung, Program Officer, Project Management Unit for Fleming Fund Project for AMR surveillance, Ministry of Health.

1. Introduction

Quality assurance (QA) primarily concerns the control of errors in the performance of tests and verification of test results. External Quality Assurance Scheme (EQAS) is an integral part of the QA program and it is a method for assessing the performance of different laboratories using external agencies such as reference laboratories (WHO, 2017).

The availability of excellent microbiological tests does not automatically guarantee reliable laboratory results. All materials, staff training and on-going competence assessment, equipment and procedures must be adequately controlled. Culture media must be tested for sterility and performance. Each laboratory must have standard operating procedures (SOPs). QA of pre-analytical, analytical, and post-analytical stages of microbiological procedures should be incorporated in SOPs. Laboratories practicing the principles of QA generate reliable, relevant, and cost-effective results, delivered timely and in the best interest of the patient.

Having a well-functioning NEQAS program is an important step towards achieving high-quality laboratory performance, through periodic monitoring of test quality, spot checking of identification tests, and isolation techniques that help to make the results obtained in different laboratories comparable (WHO, 2017). Individual laboratories can use EQA to identify problems in laboratory practices, allowing for appropriate corrective action. EQA participation will help to evaluate reliability of methods, materials, and equipment, and to evaluate and monitor training impact.

For laboratories performing public health-related testing, EQA can help to assure that results from different laboratories during surveillance activities are comparable. EQA participation is usually required for accreditation. Also, EQA participation creates a network for communication, and can be a good tool for enhancing a national laboratory network. Participating centers must **NOT** view quality assessment as a way of penalizing technicians for wrong results. Rather it is important to encourage the staff members to feel responsible for their work and to feel proud if they have done well and to stimulate them to improve if they have not done well. It can also be a tool for the organization to review whether their procedures are fit for purpose or may need review and amendment. Incorrect results are

usually not related to staff competence, but rather are procedural (reagent, equipment or method requires improvement) or change, or training issues (training process needs improving, unclear instructions, etc). Samples received for EQA testing, as well as the information shared by the EQA provider, are useful for conducting continuing education activities.

We believe that EQA schemes have great value for the assessment of quality, and rightly constructed can provide data which can form a part of a national or regional laboratory certification, accreditation, or licensing procedure. However, the limitations of EQA must also be identified and understood. There are many other potential sources of laboratory error apart from those which may be detected or controlled through an EQA scheme. It is vital that these are identified and that effective IQA procedures are in place to ensure that scarce laboratory resources of materials and personnel are used to the best advantage for the care of patients. For EQA schemes to be successful in providing independent, objective data and to act as an educational stimulus to improvement, participants must have confidence in the scientific validity of the scheme design as well as the reliability of its operations. The scientific validity will include stable, homogeneous clinical specimens (proper commutability), and valid target values; reliability involves keeping to a strict time schedule with information after analysis, structured and intelligible reports, and a rapid feedback of initial performance cumulative data system.

2. Benefits of participation in an EQA scheme

Benefits of participation in the NEQAS program are:

2.1 Provides laboratory management with an insight into their performance

2.2 Improves both national and local standards

2.3 Reveals unsuspected areas of difficulty

2.4 Acts as a check on the efficacy of internal quality control procedures

2.5 Demonstrates to colleagues and customers a commitment to quality

2.6 Provides method performance evaluation (scheme dependent)

2.7 Provides independent evidence of performance for accreditation bodies

2.8 To facilitate information exchange among participant laboratories and the EQA scheme

2.9 To stimulate performance improvement and to ensure credibility of the laboratory

2.10 To support accreditation of microbiology laboratories

3. Definition

3.1 Quality: Quality means meeting the pre-determined requirements of users for a particular substance or service. Quality includes the following:

3.1.1 Total Quality Management (TQM)

TQM evolved as an activity to improve patient care by having the laboratory monitor its work to detect deficiency and subsequently correct them.

3.1.2 Continuous Quality Improvement (CQI)

CQI seeks to improve patient care by placing the emphasis on prevention of errors and continual review and improvement in the first place.

3.1.3 Quality Assurance (QA): QA is associated with the three phases of quality assurance:

3.1.3.1 Pre- analytical

3.1.3.2 Analytical

3.1.3.3 Post –analytical

3.2 Quality control (QC): This is also called internal control where each laboratory has a program to check the quality of its own tests by continuous monitoring of test quality and comprehensive checking of all steps, from the collection of the specimen (whenever possible) to issue of the result.

3.3 Quality assurance: Defined by WHO as “the total process whereby the quality of laboratory reports can be guaranteed”.

3.4 Quality assessment: Means determining the quality of results.

3.5 AMR: is defined as resistance to antimicrobial agents in bacteria, protozoa, fungi, and viruses.

3.6 EQA: is an educational tool that allows participants to monitor, evaluate and improve their own performance.

3.7 Assigned value: Value attributed to a particular property of a proficiency test item.

3.8 Coordinator: One or more individuals with responsibility for organizing and managing all the activities involved in the operation of a proficiency testing scheme

3.9 Customer: Organization or individual for which a proficiency testing scheme is provided through a contractual arrangement

3.10 Outlier: Observation in a set of data that appears to be inconsistent with the remainder of that set.

3.11 Participant: Laboratory, organization, or individual that receives proficiency test items and submits results for review by the proficiency testing provider.

3.12 Interlaboratory comparison: Organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions

3.13 Proficiency testing (PT): Evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons.

NOTE 1: For the purposes of this International Standard, the term "proficiency testing" is taken in its widest sense and includes, but is not limited to:

3.13.1 Quantitative scheme — where the objective is to quantify one or more measurands of the proficiency test item.

3.13.2 Qualitative scheme — where the objective is to identify or describe one or more characteristics of the proficiency test item.

3.13.3 Sequential scheme — where one or more proficiency test items are distributed sequentially for testing or measurement and returned to the proficiency testing provider at intervals.

3.13.4 Simultaneous scheme — where proficiency test items are distributed for concurrent testing or measurement within a defined time period.

3.13.5 Single occasion exercise — where proficiency test items are provided on a single occasion.

3.13.6 Continuous scheme — where proficiency test items are provided at regular intervals.

3.13.7 Sampling — where samples are taken for subsequent analysis; and

3.13.8 Data transformation and interpretation — where sets of data or other information are furnished, and the information is processed to provide an interpretation (or another outcome).

3.14 **Proficiency test item:** Sample, product, artefact, reference material, piece of equipment, measurement standard, data set, or other information used for proficiency testing.

3.15 **Proficiency testing provider:** Organization which takes responsibility for all tasks in the development and operation of a proficiency testing scheme.

3.16 **Proficiency testing round:** Single complete sequence of distribution of proficiency test items, and the evaluation and reporting of results to the participants.

3.17 **Proficiency testing scheme:** Proficiency testing designed and operated in one or more rounds for a specified area of testing, measurement, calibration, or inspection. **NOTE:** A proficiency testing scheme might cover a particular type of test, calibration, inspection or several tests, calibrations, or inspections on proficiency test items.

3.18 **Robust statistical method:** Statistical method insensitive to small departures from underlying assumptions surrounding an underlying probabilistic model.

3.19 **Standard deviation for proficiency assessment:** Measure of dispersion used in the evaluation of results of proficiency testing, based on the available information

NOTE 1: The standard deviation applies only to ratio and differential scale results.

NOTE 2: Not all proficiency testing schemes evaluate proficiency based on the dispersion of results.

3.20 **Measurement of uncertainty:** Non-negative parameter indicates the magnitude of doubt of a measured value.

3.21 **Quality in Microbiology:** Quality in microbiology laboratories is about meeting the specific needs of the laboratories in the healthcare settings which provides microbiology diagnostic service and build confidence of both laboratory personnel, doctors and patients who use the results for treatment and management purposes.

4 Purpose and Scope

This guideline shall improve performances of relevant laboratory personnel in conducting clinical diagnostic microbiology and participating in the scheme and guide relevant laboratory personnel in conducting and participating in NEQAS program.

5 Objectives

The main objective of NEQAS is to establish inter-microbiology laboratory comparability of results and thereby to improve performance and strengthen the quality assurance. The specific objectives of our program are:

5.1 Primary objectives:

5.1.1 To assess the quality of laboratory performance on a nationwide basis in microbiological service

5.1.2 To provide assurance to consumers (physician and patients) on the reliability of the laboratory results

5.2 Secondary objectives:

5.2.1 To improve intra-laboratory performance of clinical diagnostic microbiology

5.2.2 To evaluate internal quality control measures

5.2.3 To identify common errors and recommend corrective measures

5.2.4 To encourage good laboratory practice, using standardized procedures, reagents and methodology in identification and antibiotic susceptibility testing in microbiology

5.2.5 To encourage implementation of quality assurance and control measures in the participating laboratories

5.2.6 Stimulate performance improvement and to ensure credibility of the laboratory

6 Stakeholders

Following agency shall be the stakeholders of NEQAS program:

6.1 Ministry of Health

The Health Care and Diagnostic Division (HCDD) under the Department of Medical Services, Ministry of Health, Thimphu, shall be responsible for the following:

6.1.1 Provide adequate funding for sustaining the program

6.1.2 Provide policy guidance related to quality assurance for the country

6.1.3 Improve the technical capacity of the QA provider

6.2 Royal Centre for Disease Control

The RCDC shall provide and support the following:

6.2.1 Administration and logistics: Assure PT samples are shipped on time with proper cold chain maintenance to the participating laboratories

6.2.2 NBCH: Provide PT samples to EIDL for preparation of panel samples

6.2.3 EIDL: The NEQAS in bacterial culture identification and AMR shall be conducted by EIDL, RCDC. This program uses qualitative schemes and expects participant laboratories to identify specific culture isolates/organisms, perform susceptibilities, Gram staining and serotyping (if applicable).

7 NEQAS program management

NEQAS program in Bhutan shall be managed and conducted by RCDC with inputs from various laboratories, national bio-banking center and administration and logistics section. NEQAS for AMR, as mentioned earlier will be coordinated by EIDL, however, the Technical Advisory Committee consisting of experts in microbiology and AMR shall provide technical guidance for conducting the program (figure 1).

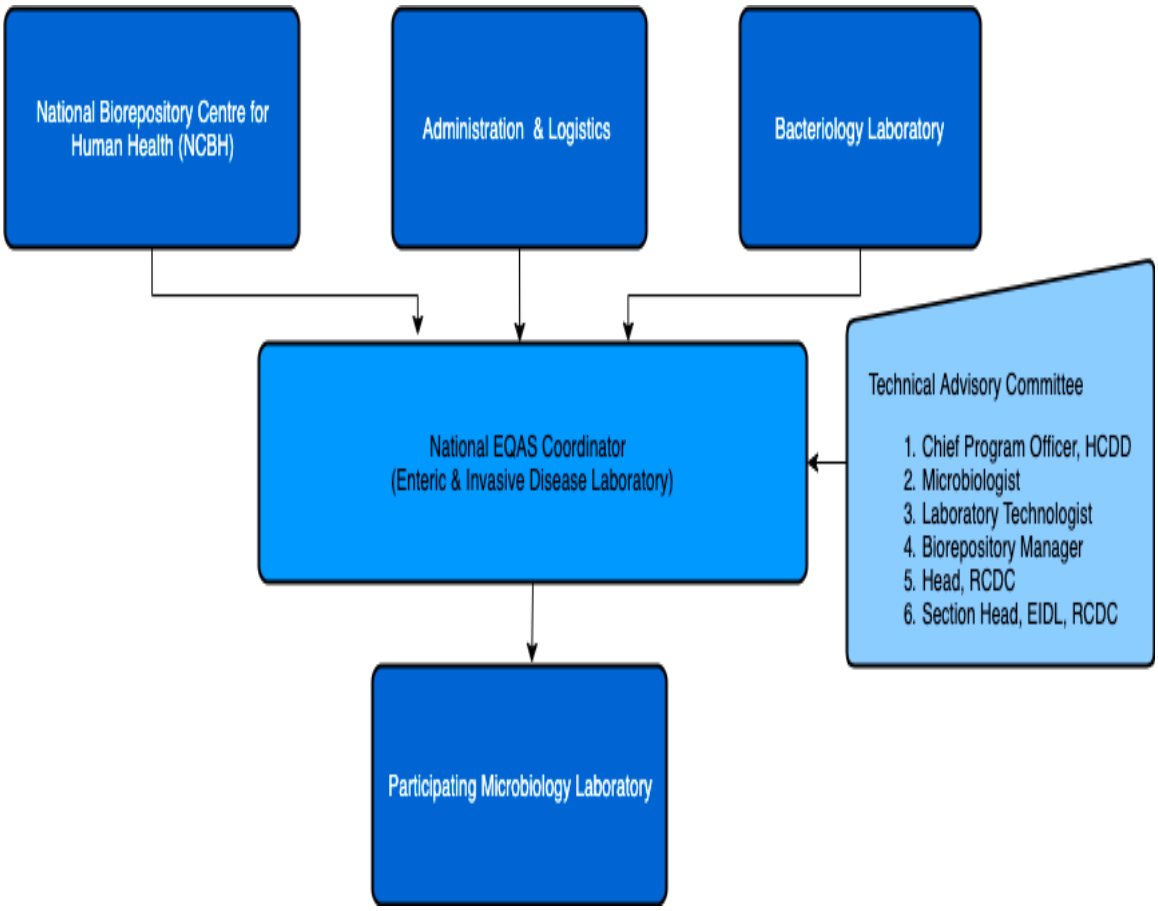

Figure 1. NEQAS program management

7.1 The Reference Laboratory (NEQAS-CC)

For the management of NEQAS, the organizing laboratory should have technical competence, necessary infrastructures, and equipment. Enteric and Invasive Disease Laboratory (EIDL) shall be the national EQAS coordinating center (NEQAS-CC) responsible for organizing and conducting all the components of NEQAS.

7.1.1 Technical requirements

7.1.1.1 General

The organizing laboratory should have the necessary expertise in the field and as it will act as a reference and will provide training and updated information on new developments in AMR. The laboratory should be acquainted with the full range of diagnostic assays used in the country and have access to adequate supply. The specimens

of the panel should be fully characterized by different assays to establish the correct diagnosis and determine SIR of the panel tested.

7.1.1.2 **Personnel**

7.1.1.2.1 The NEQAS-CC shall have adequate technical staff and managerial personnel with authority and technical expertise to coordinate and conduct NEQAS in bacterial culture identification and determine antimicrobial susceptibility. NEQAS-CC shall have adequately trained staff with minimum required qualification as below:

7.1.1.2.1.1 NEQAS Program Manager /Supervisor: Have a minimum of bachelor's degree in medical laboratory science with additional qualification/training in laboratory quality assurance (LQA) program

7.1.1.2.1.2 Laboratory Technologist: Have a minimum of bachelor's degree in medical laboratory science

7.1.1.2.1.3 Laboratory Technician: Have a minimum of certificate/diploma in laboratory science and have adequate knowledge in conducting NEQAS.

7.1.1.2.2 NEQAS-CC shall authorize above personnel to perform the following tasks related to NEQAS bacteriology and antibiotic susceptibility determination:

7.1.1.2.2.1 Select appropriate proficiency test isolates, both ATCC and isolates obtained during routine diagnosis and surveillance.

7.1.1.2.2.2 Plan NEQAS for all the health laboratories, including government and private, offering bacteriology service.

7.1.1.2.2.3 Perform a particular type of sampling.

7.1.1.2.2.4 Operate specific equipment.

7.1.1.2.2.5 Conduct measurements to determine stability and homogeneity, as well as assigned values and associated uncertainties of the measurands of the proficiency test item.

7.1.1.2.2.6 Prepare, handle, and distribute proficiency test items.

7.1.1.2.2.7 Operate the data processing system, conduct statistical analysis, and interpret results and provide feedback.

7.1.1.2.2.8 Conduct evaluation of performance of participating laboratories.

7.1.1.2.2.9 Authorize the issue of NEQAS reports and provide opinion.

7.1.1.2.2.10 Conduct periodic monitoring, onsite supervisory visit and undertake follow-up for persistent poor performing laboratories.

7.1.1.2.2.11 Periodic review and update of the NEQAS guideline.

7.1.1.2.2.12 Conduct training for the participating laboratory staff.

7.1.1.2.3 The NEQAS-CC shall maintain up-to-date records of the relevant authorization(s), competence, educational and professional qualifications, training, skills, and experience of all technical personnel. This information shall be readily available and shall include the date on which competence to perform their assigned tasks was assessed and confirmed.

7.1.1.2.4 The NEQAS-CC shall have processes in place for the education, training/retraining and skills development for each staff member involved with the operation of the NEQAS scheme. The training program shall be relevant to the present and anticipated needs of the NEQAS provider.

7.1.1.2.5 The NEQAS-CC shall ensure that staff receive the necessary training to ensure competent performance of measurements, operation of equipment and any other activities which affect the quality of the NEQAS. The effectiveness of training activities shall be evaluated and the same shall be recorded in the individual's training file.

7.1.1.3 **Facility**

7.1.1.3.1 The NEQAS-CC shall ensure that there are guidelines, protocols and Sop's for all the microbiological test, equipments, and quality data documentation.

7.1.1.3.2 The NEQAS-CC shall ensure that there is an appropriately sized laboratory, office, and adequate number of quality equipment for the operation of the NEQAS. This includes laboratory, office facilities and equipment for manufacturing, handling, calibration, testing, storage, and dispatch, for data processing, for communications, and for retrieval of materials and records.

7.1.1.3.3 The NEQAS-CC shall ensure that the environmental conditions do not compromise the proficiency testing scheme or the required quality of operations.

7.1.1.3.4 Access to NEQAS-CC laboratory shall be controlled. The management of the NEQAS-CC shall determine the extent of control based on its circumstances and have an SOP in place for the same.

7.1.1.3.5 The NEQAS-CC shall identify environmental conditions that can significantly influence the quality of the scheme and any testing and calibration carried out, including conditions that are required by relevant specifications and measurement procedures.

7.1.1.3.6 The NEQAS-CC shall ensure that performance characteristics of laboratory methods and equipment used to confirm the content, homogeneity and stability of proficiency testing items are appropriately validated and maintained.

7.2 The Participating Lab

7.2.1 **Technical requirements of participating laboratories**

7.2.1.1 **General**

The participating laboratories should have the necessary expertise in the field of bacteriology and antimicrobial resistance. The laboratory should have access to adequate supply for identification of bacterial isolates and conduct antibiotic susceptibility testing.

7.2.1.2 **Personnel**

7.2.1.2.1 Laboratory personnel in participating laboratories shall have adequate knowledge in how to process specimens, perform identification and antibiotic testing of bacterial isolates from routine diagnostic specimens.

7.2.1.2.2 They shall understand the objectives of NEQAS and their participation requirements in the program.

7.2.1.2.3 Laboratory personnel shall get the necessary training in the NEQAS program conducted by NEQAS-CC or other competent organization.

7.2.1.2.4 The laboratory personnel receiving the proficiency panel should read the accompanying documents and check the specimen condition and shall strictly abide by the NEQAS guidelines and related SOPs.

7.2.1.2.5 The laboratory personnel shall process the panel samples as soon as possible or must be stored at an appropriate temperature until testing.

7.2.1.2.6 The laboratory personnel shall report the test result to the organizing laboratory within the given deadline and upon receipt of a feedback report, the laboratory personnel shall compare these with their own test results.

7.2.1.2.7 The laboratory personnel shall follow the feedback and recommendations provided by the organizing laboratory and perform corrective actions, including identification of the source of error and perform corrective and preventive measures in case of discordance between the results of the participating laboratory and the reference result.

7.2.1.3 **Facility**

7.2.1.3.1 The participating laboratories shall ensure that there are Sop's for all the microbiological test and equipments and quality data documentation.

7.2.1.3.2 The participating laboratories shall ensure that there are adequate space and quality equipment and consumables for testing NEQAS panel samples, storage, and dispatch, for data processing and for communications.

7.2.1.3.3 The participating laboratories shall ensure that the environmental conditions do not compromise the testing scheme or the required quality of operations.

7.2.1.3.4 The participating laboratories shall identify environmental conditions that can significantly influence the quality of the scheme.

8 Designing national quality assurance schemes

8.1 Planning

8.1.1 The NEQAS-CC shall identify and plan those processes which directly affect the quality of the proficiency testing scheme and shall ensure that they are carried out in accordance with prescribed procedures.

8.1.2 The proficiency testing provider shall not subcontract the planning of the proficiency testing scheme.

8.1.3 The proficiency testing provider shall document a plan before commencement of the proficiency testing scheme that addresses the objectives, purpose and basic design of the proficiency testing scheme, including the following information and, where appropriate, reasons for its selection or exclusion:

8.1.3.1 The names and address of the proficiency testing provider

8.1.3.2 The names, address and affiliation of the coordinator and other personnel involved in the design and operation of the proficiency testing scheme

8.1.3.3 The activities to be subcontracted and the names and addresses of subcontractors involved in the operation of the proficiency testing scheme

8.1.3.4 Criteria to be met for participation

8.1.3.5 The numbers and type of expected participants in the proficiency testing scheme

8.1.3.6 Selection of the measurand(s) or characteristic(s) of interest, including information on what the participants are to identify, measure, or test for in the specific proficiency testing round

8.1.3.7 A description of the range of values or characteristics, or both, to be expected for the proficiency test items

8.1.3.8 The potential major sources of errors involved in proficiency testing offered

8.1.3.9 Requirements for the production, quality control, storage, and distribution of proficiency test items

8.1.3.10 Reasonable precautions to prevent collusion between participants or falsification of results, and procedures to be employed if collusion or falsification of results is suspected

8.1.3.11 A description of the information which is to be supplied to participants and the time schedule for the various phases of the proficiency testing scheme

8.1.3.12 For continuous proficiency testing schemes, the frequency or dates upon which proficiency test items are to be distributed to participants, the deadlines for the return of results by participants and, appropriately, the dates on which testing, or measurement is to be carried out by participants.

8.1.3.13 Any information on methods or procedures which participants need to use to prepare the test material and perform the tests or measurements.

8.1.3.14 Procedures for the test or measurement methods to be used for the homogeneity and stability testing of proficiency test items, procedures for the preparation of proficiency test items and, where applicable, to determine their biological viability.

8.1.3.15 Preparation of any standardized reporting formats to be used by participants.

8.1.3.16 A detailed description of the statistical analysis to be used.

8.1.3.17 The origin, metrological traceability, and measurement uncertainty of any assigned values.

8.1.3.18 Criteria for the evaluation of performance of participants.

8.1.3.19 A description of the data, interim reports, or information to be returned to participants.

8.1.3.20 A description of the extent to which participant results, and the conclusions that will be based on the outcome of the proficiency testing scheme, are to be made public; and

8.1.3.21 Actions to be taken in the case of lost or damaged proficiency test items.

8.1.4 The proficiency testing provider shall have access to the necessary technical expertise and experience in the relevant field of testing, calibration, sampling, or inspection, as well as statistics. This may be achieved, if necessary, by establishing an advisory.

8.1.5 Technical expertise shall be used, as appropriate, to determine matters such as the following:

8.1.5.1 Planning requirements as listed in 6.1 and 6.2.

8.1.5.2 Identification and resolution of any difficulties expected in the preparation and maintenance of homogeneous proficiency test items, or in the provision of a stable assigned value for a proficiency test item.

8.1.5.3 Preparation of detailed instructions for participants.

8.1.5.4 Comments on any technical difficulties or other remarks raised by participants in previous proficiency testing rounds.

8.1.5.5 Provision of advice in evaluating the performance of participants.

8.1.5.6 Comments on the results and performance of participants as a whole and, where appropriate, groups of participants or individual participants.

8.1.6 Access to biological material

The organizing laboratory should have access to biological material in enough quantity to prepare the proficiency panels. The NBCH shall provide biological materials selected randomly from the biorepository.

8.1.7 Participation in the scheme

Participation is mandatory for all the laboratories with microbiology facilities and for all the proficiency cycles. The concerned laboratory shall receive prior notice from the organizers about the scheme. Those laboratories who intend to enroll in the NEQAS shall submit a duly filled 'Registration form' and should make a request through the relevant QA officials. Each microbiology laboratory shall receive and follow the latest edition of the manual on "NEQAS in AMR". Participation and sample issues will be free of charge to all the participating laboratories.

9 Process for conducting NEQAS program

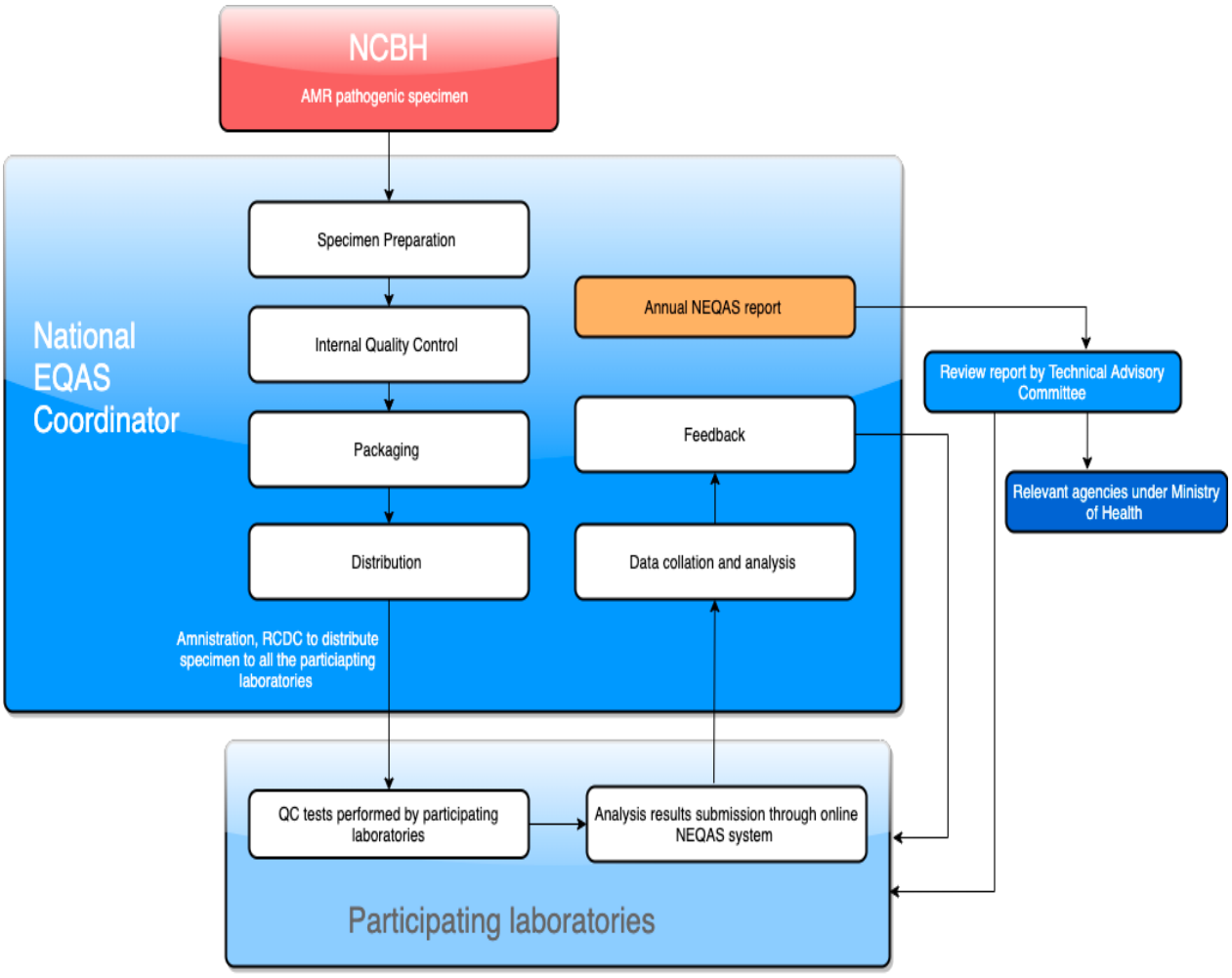

Figure 2: Overall process of conducting NEQAS for AMR pathogen detection

9.1 Components of bacteriology NEQAS

9.1.1 Proficiency testing

According to ISO/IEC Guide 43-1:1997: “Proficiency testing schemes (PTS) are inter-laboratory comparisons that are organized regularly to assess the performance of analytical laboratories and the competence of the analytical personnel”. Proficiency testing (PT) scheme is the process of evaluating performance of a laboratory by a third-party laboratory through analysis of unknown samples. Proficiency testing scheme (PTS) has been used by laboratories for many years. It is the most employed type of EQA laboratories, as it can assess many laboratory methods. Proficiency testing is available for most of the commonly performed laboratory tests, and covers a range of chemistry, hematology, microbiology, and immunology testing.

9.1.2 Benefits of participating in PTS

9.1.2.1 Participating in bacteriology PTS gives the laboratories an opportunity to assess and demonstrate the reliability of the test result generated.

9.1.2.2 It reveals the strengths and weaknesses in laboratory performance in bacteriology and its antibiotic susceptibility testing (AST) and whether the laboratory requires corrective actions.

9.1.2.3 Positive evaluation by the reference laboratory validates the technical competency of the laboratory and increases its confidence in the results generated in their laboratory. Inter laboratory comparison of their performances can be made; this would provide opportunities to learn from each other.

9.1.3. Process

In the proficiency testing process, the participating laboratories shall receive samples from the NEQAS-CC. The NEQAS-CC shall be the National Reference Laboratory (NRL) for bacteriology. Currently Enteric and Invasive Disease Laboratory (EIDL) under Royal Centre for Disease Control (RCDC) is recognized as the coordinating center for NEQAS (in Bacteriology) in the country. The process begins with random samples being obtained from the National Bio-banking Centre for Health (NBCH). The specimens will be identified by the NEQAS-CC and aliquoted. The aliquoted samples will be distributed to the participating

laboratories (PL) under proper transport conditions. The PL shall analyze the samples and submit their results to the NEQAS-CC. The results submitted shall be evaluated and analyzed, and the PL are provided with information about their performance and how they compared with other participants. The participating laboratories use the information regarding their performance to make appropriate changes and improvements (Figure 3).

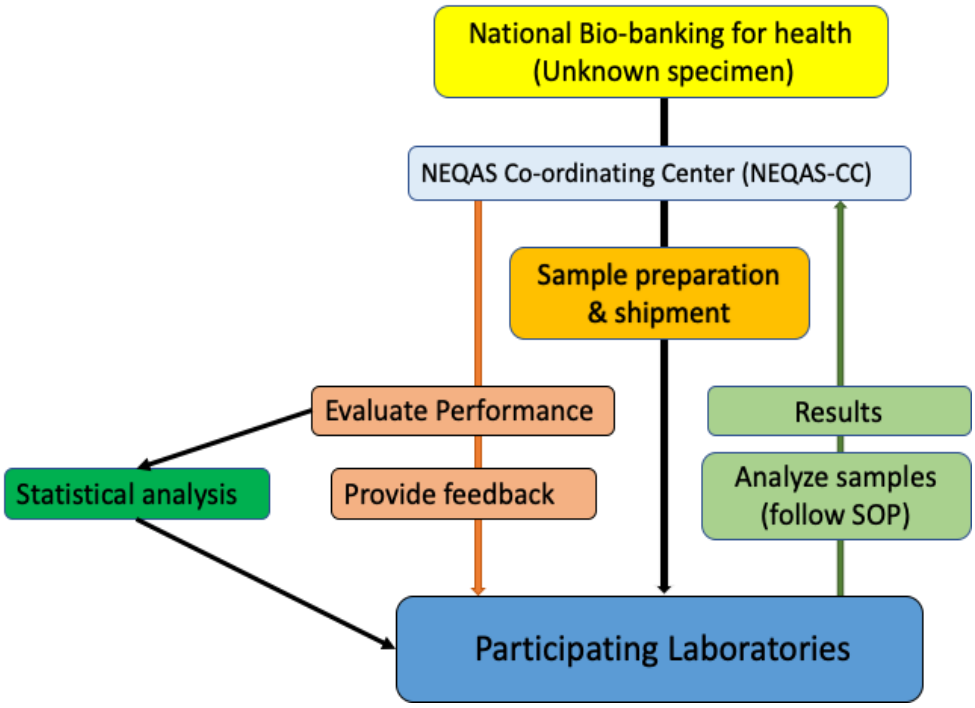

Figure 3: Process of proficiency testing scheme.

9.1.4. **Preparation of PT samples**

9.1.4.1 Proficiency testing providers should make every effort to produce samples that exactly mimics the usual patient samples.

9.1.4.2 In addition to ATCC strains, unique isolates obtained from routine samples will be screened and confirmed using the state-of-the-art technologies before submitting to the biorepository. Results of antimicrobial susceptibility of each isolate in the biorepository will be recorded for future reference.

9.1.4.3 For preparing the proficiency material, the isolate of choice by the organizer will be retrieved from the biobank and an additional identification/antimicrobial susceptibility test would be carried out for confirmation before packaging for shipment.

9.1.5. **PT schedule and distribution of samples**

For accreditation in bacteriology and AST, NEQAS-CC requires the PL to participate in two PTs per year unless a challenging situation occurs.

9.1.6. **Timeframe**

9.1.6.1 The participants are expected to receive the PT panels, run analysis, and should submit the result to the organizer within 1 month. i.e., the day RCDC ships-out the PT samples will be considered as day one (D1) of the one month.

9.1.6.2 D30 will be the last day/ closing day for result submission by the participating labs, beyond which no results will be accepted, and the center will be labelled as a “Non-responder”.

9.1.6.3 One week after D30, the organizer shall evaluate the result and provide an immediate report, ‘preliminary report’, to those centers which have reported discordant results. A troubleshooting guide or recommendations will be enclosed with the preliminary report to solve the presumptive problem immediately.

9.1.6.4 For the next 30 days (D60), the reports will be collated and analyzed by the organizing team and a final report will be prepared and shared with the participating centers. Thus, one Proficiency cycle will be completed in 2 months’ time excluding the time taken by the organizer in preparing the PT materials for distribution. See figure 4 below.

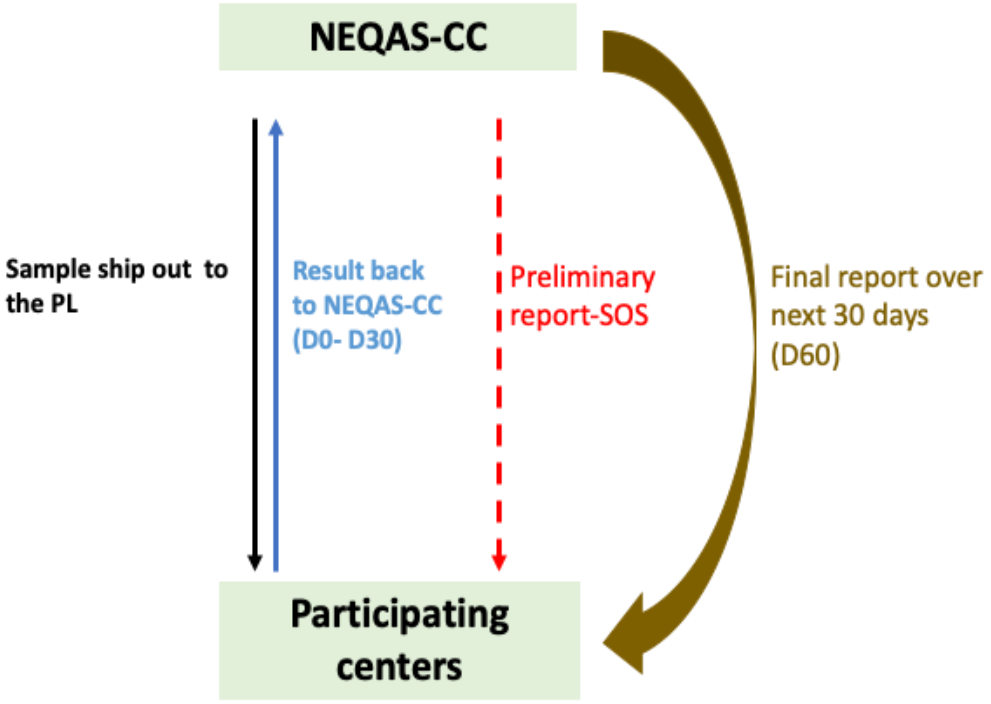

Figure 4: Proficiency testing cycle

9.1.7. **Laboratory analysis**

Once received, the PT samples at the participating laboratories should be inspected for shipment related damages. The conditions of the container and the contents should be noted in the form (Table 1). All the necessary information regarding samples should be fed into the Laboratory Information Management System (LIMS). The concerned laboratory supervisors should be notified of the samples for analysis and prepare accordingly as per the instructions given. After preparation, the samples should be integrated with the normal routine samples and should be tested in the same manner without any special attention for the PT samples.

9.1.8 **Result submission**

After the samples are analyzed, the concerned laboratory officials should submit the results to the concerned Laboratory Supervisors and the results of both PT and QC samples are submitted to the NEQAS-CC for evaluation. Study results should be reported online/emails/paper based/ any possible electronic media platform.

9.1.9 **Assessment of PT Laboratory Performance**

PT instructions must be followed strictly with all the paper works completed. The result should be submitted within the prescribed deadline. All PT results, as well as corrective actions, should be well documented. The NEQAS-CC receives the report form from the PL, reviews them and assigns each a mark or grade. The assessment considers the following information:

i. expected results on the required tests
ii. results, observations, and feedback from the participating laboratories

All these sources are considered because unexpected events may prevent laboratories from achieving the expected result.

9.1.9.1 **Scoring**

Scoring areas are sections of the scheme results to which the NEQAS-CC assigns marks. Each scoring area corresponds to a critical decision point or an analytical test that the participating laboratory performed. Depending upon the specimens in the scheme and the tests conducted, the scoring areas may include one or the following:

i. Culture and identification
ii. Serotyping
iii. Antimicrobial results and reporting

The scores shall be awarded for correctly identifying the isolate (genus, species), and the concordance of the antimicrobial susceptibility zone diameters to that of the NEQAS-CC. This is the range for interpretation of antimicrobial susceptibility recommended by the vendors. The results submitted by the PL should be collected and collated by the NEQAS-CC.

9.1.9.2 **Data Management**

Data from EQA results shall be entered into the Microsoft excel and electronic databases and backed-up. All results, data analysis, evaluations and reports shall be archived.

9.1.9.3 **Performance indicator**

The z-score is a useful tool generated during a PT study that should be used as a performance indicator in bacteriology. A z-score is a measure of the deviation of the result (Xi) from the assigned value (X) for that determinant and are calculated as:

$$z = (Xi - X)/\sigma$$, where σ is a standard deviation.

PT Sample results are evaluated using some form of parametric or nonparametric statistics to determine the mean and standard deviation. Determination of the method used to calculate the z-score should be determined by the PT Provider. Once a z-score has been determined, the performance is evaluated using a scale.

9.1.9.4 **Failed PT study**

Proficiency test acceptance limits shall be used to determine the pass/fail evaluation of PT. For PT to be acceptable, a laboratory should score 80% to receive a score of "Pass". Error of more than 20% will receive a score of "Fail". Failure to meet the semi-annual schedule for PT testing is also regarded as a failed study.

The NEQAS-CC shall have a procedure where, if a number of PL cannot isolate the target organism that consideration shall be given to the participants had the error has occurred at the reference laboratory i.e. a mix up of samples or the quality of the PT sample being sub-optimal and the PL performance shall not be scored. If any of the laboratories fails a PT study, the organizing laboratory officials will initiate the investigative process.

9.1.9.5 **Failing two PT studies**

If the PL fails both the most recent PT studies, the bacteriology laboratory will not be accredited. In this case, a lab may participate in a Supplemental PT Study for demonstrating Corrective Action and meet the requirements for initial accreditation.

The NEQAS-CC shall have a procedure where, if a number of PL cannot isolate the target organism that consideration shall be given to the participants if the error has occurred at the reference laboratory i.e. a mix up of samples or the quality of the PT sample being sub-optimal and the PL performance shall not be scored. Under these circumstances, the Laboratory officials initiate an investigation on the cause of failure and document the

corrective action taken. The Organizing Laboratory provides recommendations and submit both the results of the investigation and the corrective action measures taken to the NEQAS-CC.

10 On-site evaluation

A periodic visit by evaluators for on-site laboratory assessment is a type of EQA that has been used when other methods of EQA are not feasible or effective. Again, this method has most frequently been employed for assessment of sites performing bacteriology and antibiotic susceptibility testing. On-site evaluation for the purpose of EQA should be conducted by the NEQAS-CC or other competent health laboratory. On-site evaluation can be used together with retesting and rechecking schemes to provide more information about performance. The on-site evaluation can be a valuable tool to:

i. Obtain a realistic picture of laboratory practices by observing the laboratory under routine conditions to check that it is meeting quality requirements and provide information for internal process improvement.
ii. Measure gaps or deficiencies-learn “where we are”.
iii. Assist the laboratory in collecting information for planning and implementation of training, monitoring, and corrective actions.

10.1 Long term evaluation

If the laboratory performance during a PT assessment is unsatisfactory then corrective action should be made:

10.1 After two consecutives, questionable, or marginal results.

10.2 If the results for any analysis show a clear trend towards unsatisfactory performance over three or more rounds; or

10.3 If a clear bias is observed in the results

11 Limitations of PT

11.1 It is not appropriate to use PT as the only means for evaluating the quality of a laboratory.

11.2 PT results are affected by variables such as preparation of the sample, matrix effects, clerical errors, selection of statistical methods of evaluation, and peer group definition.

11.3 PT will not detect all problems in the laboratory, particularly those that address the pre- and post-examination procedures.

11.4 A single unacceptable result does not necessarily indicate that a problem exists in the laboratory.

12 Preparation of annual NEQAS performance report

The EQA annual report is a summary of all the scores of all the schemes in a single year. This includes the correct or expected scores and the scores of all PL. Individual reports showing the participant's results as well as the statistical analysis of the survey are produced for the survey. The report includes the participant's participation and analytical performance scores.

12.1 **Web reports:** A Portable Document Format (PDF) copy of the report for each survey is posted to the web server used for data entry. Reports on the web server are protected by the same level of security as is used for result entry. Registered web users are informed by email of the availability of new reports.

12.2 **Non-web reports:** A copy of the report will be sent to the participating laboratories. Participants should ensure that their reports are retained and are available to staff. Duplicate copies of reports can be provided if necessary.

13 Dissemination of the NEQAS report

Report should be dissimilated within a month of data received from the PL. Individual reports will be produced for each participant following each distribution.

14 Confidentiality

Each participating laboratory shall be allocated with a confidential code name. A coded specimen shall be shipped to all the participating laboratories by mail from the organizers. Each participant shall receive a confidential individual hard copy on their performance throughout the year. The reports, scores and feedback sent to individual participants shall be maintained with strict confidentiality. The EQA report is an individual feedback report customized for each laboratory so that it contains only that laboratory's scheme scores. it also includes an explanation of the grading criteria and technical commentary on any discrepancies compared with the acceptable results. The receipt laboratory can use the information as a review of its methods and guide for improvements.

Annex 1 NEQAS Lab action flow chart

NEQAS lab actions in **green boxes**, participants actions in **orange boxes**

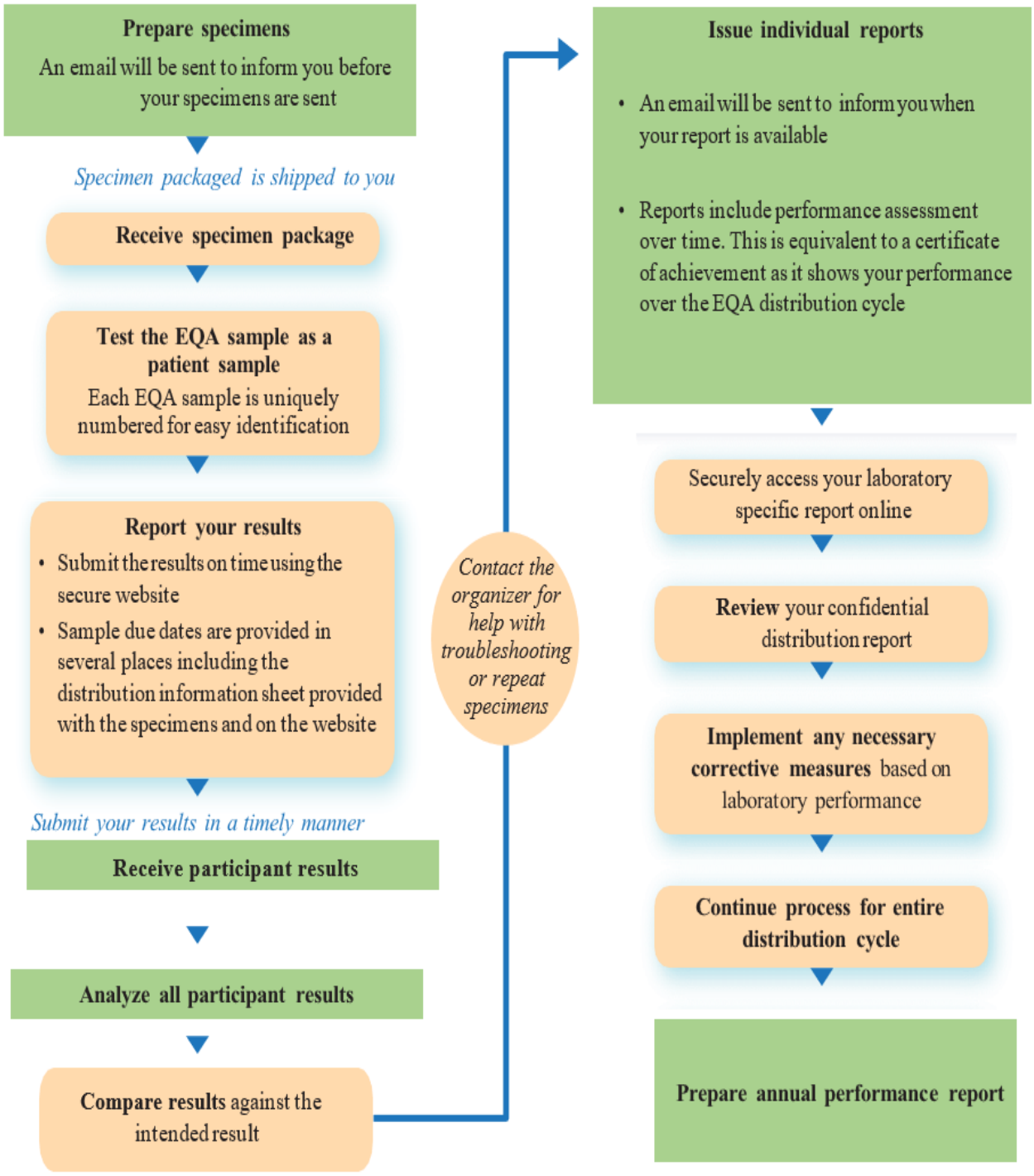

Figure 5. NEQAS Lab action flow chart

Annex 2 Packaging

Proficiency test items should be classified as Category B substances. Category B infectious substances have the proper shipping name “Biological Substance, Category B” and the identification number UN 3373. Packaging Category B infectious substances must be tripled packaged and compliant with IATA Packing Instruction 650 detailed in Figure 6. Labeling The outer container of all Category B infectious substance packages must display the following on two opposite sides:

- Sender’s name and address
- Recipient’s name and address
- The words “Biological Substance, Category B”
- UN 3373 label
- Class 9 label, including UN 1845, and net weight if packaged with dry ice

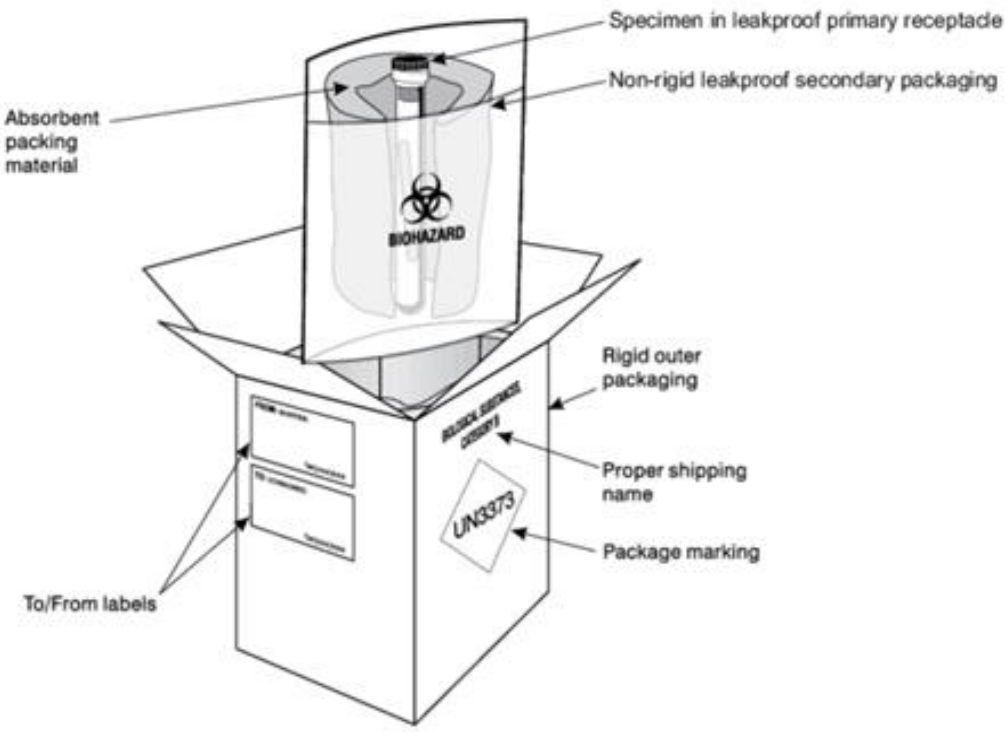

Figure 6. Triple package for transporting biological substances, category- B

Notes:

1. Materials should be packed in a cold chain box to prevent breakage and maintain proper temperature during transportation.
2. Vials containing lyophilized material should be packed with cushioning material to prevent breakage during transport, and with enough absorbent material to absorb the spillage.

Shipment protocol:

The shipment of PT specimens must comply with the IATA regulations. A courier agent should be informed regarding the transportation of the proficiency test specimen to determine optimal arrangements for dispatch and timely delivery of the items. The courier agent should have fair knowledge regarding the IATA.

Annex 3 General Instructions for the participant

1) The cultures should be tested immediately upon receipt. If delayed, store the samples at 2 to 8ºC to maintain sample quality.

   Note: The culture sample should not be stored for a longer period as prolonged storage will dry up the media and bacterial viability will be lost.

2) The culture specimens received should be incorporated into the laboratory routine, handled, and tested in the same way as routine clinical specimens. so that the survey is representative of routine laboratory performance, as highlighted in the following examples:
   a) The most expert member of staff should not always perform the survey unless there are no other staff members available. The labs should make every effort to ensure all staff members who normally work in the lab participate in proficiency testing over a given time frame. This can also then be used as part of staff ongoing competency evaluation. It is a useful indicator of whether you have procedural drift (staff members deviating from documented procedures).
   b) There should be no collaboration between different staff members unless the results indicate that this would be the case with a similar clinical sample.
   c) The same specimen should not be tested multiple times unless the results indicate that this would be the case with a similar clinical specimen.
   d) There should be no collusion with other institutions.
3) Universal safety precautions must be strictly adhered when performing the test.
4) Complete report of the test performed shall be sent to the organizer via email or post.

Annex 4 Process/step on the submission of NEQAS-CC Results

1. Refer to the summarized information of the NEQAS-CC Sample enclosed with the following details:

    - Title of the program
    - Cycle number
    - Lot number
    - EXP (Expiration Date)
    - Calendar of Submission Due Dates
    - Stability Conditions-
        - ✓ Once received, these samples will be stable until the expiration date when stored unopened at 2-8C.
        - ✓ Once sample is reconstituted, the sample will be viable for 2 days when stored tightly capped at 2-8C.

2. Testing of Samples and Submission of Results

2.1 Prioritize testing of the sample upon receipt. Submit corresponding results before the given due date.

2.2 Sample Bottle/vial Number corresponds to the chronological order of the bottles/vials to be tested per month. Submission due date of results is also indicated in the bottle/vial.

2.3 NEQAS samples nos. 1, 2, 3, and 4 (July, Aug, Sept, Oct) are identified as late samples, as they are beyond the “sample due date” (indicated in their corresponding bottles).

2.4 However, you are still required to test all late sample bottles separately, preferably one week apart and submit their corresponding results.

2.5 Late Data received after the “end of cycle” will no longer be accepted.

Table 1. NEQAS Panel Reporting Form

ཀཡལ་ལུན་འཚང་གཞུང་ཁང་། གསོ་བའི་ལུན་ཁག། མི་སངས་གསོ་བའི་ཐད་ཀར་ཨེན་ཇི་ཨེ།

ROYAL GOVERNMENT OF BHUTAN
MINISTRY OF HEALTH
DEPARTMENT OF PUBLIC HEALTH
Royal Centre for Disease Control

NEQAS Panel reporting form

Result sheet	Received date:.....
Bacterial Identification and Susceptibility testing	Test date:……. Test completed on: ……
Sample details: Panel round #: Panel sample code: Laboratory sample ID:	
Panel sample condition: ..**……**	

1. Media used for culture and Identification

Media inoculated *E.g., MacConkey agar*	Media Brand *Eg.Oxoid*	Expiry date *Day/Month/ Year*	Atmosphere & Temperature. *Eg.Co_2 Candle Jar,37°C*	Colony Characteristics Eg.Gray, moist.1mm

2. Method used in Antimicrobial Susceptibility Testing (please tick)

□ 2.1 Kirby Bauer □ 2.2 MIC (E-test) □ 2.3 MIC (Automated instrument)

□ 2.4 Others (please specify)…**……**

3. Antibiotic(brand) used in Susceptibility Testing are from (please tick):

□3.1 Difco □3.2 Oxoid □3.3 Biomerieux □3.4 Mast

□3.5Others (please specify)**……..**

4 Identification results:

4.1Sample No…….. Genus…… Species……..Group**……**

5.Susceptibility results

Antibiotics (name in full)	Lot/Exp date of each drug	Zone diameter(mm) or MIC(µg/ml)	Interpretation (please circle)

Sample No……..			
			S I R NS
			S I R NS
			S I R NS
			S I R NS
			S I R NS
			S I R NS
			S I R NS
			S I R NS
			S I R NS
			S I R NS

Comments:……

……

……

S: susceptible I: intermediate R: resistant NS: non susceptible

Name of Analyst: ……Position: ……

Name of Hospital:……Mobile no.:……E-mail:……

Annexure 5: PT Sample condition reporting form

Initial task upon receipt of PT sample

- Document the date and time the PT sample received.
- Assign an accession number to be used as PT sample identification in the laboratory.
- Verify that the identification on the testing request form matches the identification on the PT sample.
- Examine the PT sample visually to evaluate for acceptability.
- Review and evaluate the test request for suitability.
- Determine the suitability, with respect to the test(s) ordered, of the transport conditions, including the following:
    - Transport medium or preservative for the sample
    - Temperature of sample upon receipt
    - Length of time between sample collection and receipt
    - Transport container intact, i.e., no leaks or cracks.

Actions for when PT samples are rejected

- If the unacceptable sample can be replaced, notify the requesting provider.
- Document the reason for the sample unacceptability and request another PT sample.
- Do not discard the sample until the provider has confirmed that another can be provided.
- If a repeat PT sample is not available, document the problem and proceed with the request test if possible.

Annexure 6: Instruction for Opening and Reviving Lyophilized Reference Strain Culture

Lyophilized cultures are supplied in vacuum-sealed vials/ampoules. Care should be taken in opening the vial/ampoule. All instructions given below should be followed closely to ensure the safety of the person who opens the vial/ampoule and to prevent contamination of the culture.

- Upon receipt of samples ensure that the content is dry and protected from light. The test strains represent bacterial organisms in the form of freeze-dried vials.
- Keep samples in the dark at 2-8 °C until testing.
- All testing should be performed in a BSL2 level laboratory or in a biosafety cabinet class II.
- Check the sample numbers on the label on the vials/ampoules.
- Disinfect the lid of the vial with alcohol dampened gauze or cotton.
- Aseptically open the vial.
- Slowly add 1.0 mL tryptic soy broth (or other sterile microbiological diluents like 0.85% Nacl, Nutrient broth) using sterile Pasteur pipette and mix carefully to avoid creating aerosols until the pellet has dissolved.
- Leave for 10 minutes.
- Transfer the content to one or more suitable solid and/or liquid media.
- Incubate the inoculated medium at appropriate conditions for several days.
- Autoclave or effectively disinfect the used Pasteur pipette and the remains of the original vial before discarding.

Notes:

- Cultures should be grown on media and under conditions as per the microbiological procedure for sample identification.
- Cultures may need at least one subculturing before they can be optimally used in experiments.
- Unopened vials/ampoules should be kept in a dark and cool place.

Annexure 7. NEQAS Registration Form

དཔལ་ལྡན་འབྲུག་གཞུང་། གསོ་བ་ལྡན་ཁག། མི་མང་གསོ་
བའི་ལས་ཁུངས།

ROYAL GOVERNMENT OF BHUTAN
MINISTRY OF HEALTH
DEPARTMENT OF PUBLIC HEALTH
Royal Centre for Disease Control

National External Quality Assessment Scheme (NEQAS) in Antimicrobial Resistance (AMR)

Registration Form

For EQAS use only (shall be filled by the organizer)

Name of laboratory/hospital: ____

Laboratory confidential code____

A. LABORATORY INFORMATION

Name and address of the identified person to whom the test panels shall be dispatched. The test report and feedback will also be sent to this address

Name of the focal person: ____

Designation: ____

Contact number: ____

Email ID: ____

NEQAS participation: ☐ New participation ☐Old participation

Please check which program your laboratory would like to enroll

☐AMR in Bacteriology ☐Parasitological ☐Gram staining

B. POSTAL ADDRESS

Detailed address with postal code of the participating laboratory for shipping the test panel

____

____

____

____

This laboratory agrees to abide by the guidelines and conditions of participation of the NEQAS scheme

Name and Signature: ____________________________________

Designation: ____

Date: ____

Table 2. Sample Condition Reporting From

Si. No	Date of PT sample received	Name of the PL	PT sample code	Criteria for rejection	Action	Performed by (Name/Sign)

Reference

1. WHO (2017), Content Sheet 10-1: Overview of External Quality Assessment (EQA).
2. Zdzislaw Kolasinski (2010), Guidance in Implementation of Proficiency Testing and Performance evaluation studies, Zdzislaw Kolasinski (zkolasin@sfwmd.gov) State Laboratory ID: E46077 Analytical Services Division Restoration Sciences Department South Florida Water Management District.
3. Guidelines for national external quality assessment scheme for STI/TTIs serology. Public Health Laboratory Services. Ministry of Health, Thimphu, Bhutan. First edition, 2013
4. ISO/IEC 17043, First edition, 2010-02-01, Conformity assessment — General requirements for proficiency testing
5. https://ukneqasmicro.org.uk/images/pdf/DOC.0427.pdf,