

THE RAPID SYPHILIS TEST TOOLKIT IMPLEMENTATION 2

Quality Management System Guide for Rapid Syphilis Testing



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Quality Management System Guide for Rapid Syphilis Testing

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Glossary

Definitions for the following terms are the internationally accepted definitions supplied by the Clinical and Laboratory Standards Institute (CLSI) in its **Harmonized Terminology Database**.

It is publicly available at: [http://www.clsi.org/AM/Template.cfm?Section=Harmonized Terminology Database](http://www.clsi.org/AM/Template.cfm?Section=Harmonized_Terminology_Database)

Accuracy (of measurement) Closeness of agreement between a measured quantity value and a true quantity value of a measurand.

Agreement The proportion of specimens where results obtained using a new test and those obtained using an imperfect standard agree.

Algorithm A set of rules for solving a problem in a finite number of steps, as for finding the greatest common divisor.

Audit A planned, independent, and documented assessment to determine whether agreed-upon requirements are being met.

Biohazard A biological agent or condition that constitutes a hazard to human beings or their environment.

Biosafety Cabinet Hood designed specifically to contain microorganisms. It is designed to protect workers, the environment, and laboratory consumables from contamination. It can also be designed to use small amounts of chemicals and to keep products in the hood clean.

Calibration Operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication.

Characterization (for a reference material) Determination of one or more physical, chemical, biological, or technological property values that are relevant to its intended end use. The characterization process provides the values for the properties to be quantified.

College of American Pathologists Accreditation Program: An internationally recognized program based on the CAP Laboratory Accreditation Standards that helps laboratories achieve the highest standards of excellence to positively impact patient care.

Corrective action Action taken to eliminate the cause(s) of existing problems, defects, or any other undesirable situation in order to prevent recurrence.

Document Any recorded item of a factual or informative nature, either paper or electronic; written or electronically generated information and work instructions.

Dried Tube Specimen Dried serum or plasma patient specimen that are cold chain independent, for use as a quality control sample or proficiency testing program.

Dried Blood Spots Blood collected on filter paper and dried for transport and testing for HIV and other diseases. Can also be used as a quality control measure.

External Quality Assessment Evaluation of the laboratory's performance on examination of samples of external origin for the purposes of determining adequacy of the laboratory's pre-examination, examination, and post-examination activities; The main object is to establish between-laboratory and between-instrument comparability that is, if possible, in agreement with a reference standard (where one exists). External quality assessment schemes may be regional, national, or international. It is sometimes also referred to as "proficiency testing," especially when the external agency is a regulatory agency. Interlaboratory comparisons and other performance evaluations may extend throughout all phases of the testing cycle, including interpretation of results; determination of individual and collective laboratory performance characteristics of examination procedures by means of interlaboratory comparison.

External Quality Control External quality control and assurance or proficiency testing is the evaluation of analytical performance that includes a sample for which the analyst does not know the expected measurement result.

False-negative Negative test result for a patient or specimen that is known or subsequently proved to be positive for the condition or constituent in question.

False positive A positive test result for a disease or condition when the disease or condition is not present. A positive test result for a patient or specimen that is known or subsequently proved to be negative for the condition or constituent in question.

Form A paper or electronic document on which the results from the performance of a procedure or other information are captured, after which it becomes a record.

Good Laboratory Practice Embodies a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, recorded, reported, and archived. GLP helps assure regulatory authorities that the data submitted are a true reflection of the results obtained during the study, and therefore can be relied upon when making risk/safety assessments.

Good Clinical Practice An international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects.

Instructions For Use Information supplied by the manufacturer with an in vitro diagnostic medical device concerning the safe and proper use of the reagent or the safe and correct operation, maintenance, and basic troubleshooting of the instrument.

Integrity A measure of functionality

Interference Artifactual increase or decrease in apparent concentration or intensity of an analyte due to the presence of a substance that reacts non-specifically with the measurement system.

Internal quality control The evaluation of analytical performance that includes quality control samples for which the analyst knows the expected measurement result.

Negative Predictive Value The likelihood that an individual with a negative test result does not have the disease, or other characteristic, that the test is designed to detect. This is equal to the number of true-negative cases divided by the sum of true-negative plus false-negative cases.

Point-of-care Testing performed in an alternate site, outside a central laboratory environment, generally nearer to, or at the site of, the patient.

Positive Predictive Value The likelihood that an individual with a positive test result has a particular disease, or characteristic, that the test is designed to detect; This varies with the prevalence of the disease in the population tested.

Preventive action Action taken to eliminate the cause(s) of potential problems, defects, or any other undesirable situation in order to prevent occurrence.

Preventive maintenance Scheduled periodic work on a piece of equipment that is not a result of malfunction or failure and is intended to avert such failure.

Procedure Specified way to carry out an activity or a process.

Process Set of interrelated or interacting activities which transforms inputs into outputs.

Process Control A series of quality control processes required to produce a result from a patient specimen and to handle/manipulate/transport the specimen.

Process Improvement Part of a process management focused on reducing variation and improving process effectiveness and efficiency.

Proficiency Testing A program in which multiple samples (proficiency panel) are periodically sent to members of a group of laboratories for analysis and/or identification, in which each laboratory's results are compared with those of other laboratories in the group and/or with an assigned value, and reported to the participating laboratory and others.

Qualitative Characterization applied to laboratory tests that detect and/or identify a particular analyte, constituent, or condition. When used to describe a test, means a test that produces a result that is descriptive rather than numerical. For example, a rapid syphilis test might generate a result of 'positive' or 'negative'.

Quality Assurance Part of quality management focused on providing confidence that quality requirements will be fulfilled. The practice that encompasses all procedures and activities directed toward ensuring that a specified quality of product is achieved and maintained. In the testing environment, this includes monitoring all the raw materials, supplies, instruments, procedures, sample collection/transport/storage/processing, recordkeeping, calibrating and maintenance of equipment, quality control, proficiency testing, training of personnel, and all else involved in the production of the data reported practice that encompasses all procedures and activities directed toward ensuring that a specified quality of product is achieved and maintained. In the testing environment, this includes monitoring all the raw materials, supplies, instruments, procedures, sample collection/transport/storage/processing, recordkeeping, calibrating and maintenance of equipment, quality control, proficiency testing, training of personnel, and all else involved in the production of the data reported.

Quality Control The set of procedures undertaken in a laboratory, or clinic, for the continuous assessment of work performed and evaluation of tests to decide whether these are reliable enough for release of results to the requesting health care provider and patient. Quality Control includes testing control materials, charting the results and analyzing them to identify sources of error, and evaluating and documenting any remedial or corrective action taken as a result of this analysis.

Quality Management System Management system to direct and control an organization with regard to quality. A quality management system typically includes the organizational structure, resources, processes, and procedures needed to implement quality management; These principles include categories such as Documents and Records, Organization, Personnel, Equipment, Purchasing and Inventory, Process Control, Information Management, Occurrence Management, Assessments—External and Internal, Process Improvement, Customer Service, and Facilities and Safety.

Quantitative A characterization applied to laboratory tests that give results expressing a numerical amount or level (concentration) of an analyte in a specimen.

Recombinant Artificial methods of producing DNA (synthetic DNA and proteins).

Reconstitution Restoring (a liquid in concentrated or powder form) to normal strength by adding water.

Record Document stating results achieved or providing evidence of activities performed.

Reference method An exactly defined technique that is used in association with an internationally agreed reference preparation to provide sufficiently precise and accurate data for assessing the validity of other methods.

Sensitivity (of a measuring system) Quotient of the change in an indication of a measuring system and the corresponding change in a value of a quantity being measured. In clinical settings, this is defined as the proportion of patients with a well-defined clinical disorder (or condition of interest) whose test values are positive or exceed a defined decision limit (i.e., a positive result and identification of the patients who have a disease).

Shelf-life Period of time until the expiration (expiry) date.

Specification A document that specifies, in a complete, precise, verifiable manner, the requirements, design, behavior, or other characteristics of a system or component, and often, the procedures for determining whether these provisions have been satisfied.

Specificity The ability of a test or procedure to correctly identify or quantify an entity in the presence of interfering phenomena/influence quantities. In a clinical setting, it is the percentage of subjects without the target condition (as determined by the diagnostic accuracy criteria) whose test values are negative.

Stability Capacity for a product to retain its composition, characteristics, and properties during specified conditions.

Standard Operating Procedure A set of standardized and documented procedures that form the basis of any specified action.

Titre The dilution of the antibody at which a specified percentage of the analyte is bound under defined conditions.

Traceability (metrological) Property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty. The ability to trace the history, application, or location of an entity by means of recorded identifications.

Validation Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled. The World Health Organization (WHO) defines validation as “the action (or process) of proving that a procedure, process, system, equipment, or method used works as expected and achieves the intended result”.

Verification Confirmation, through the provision of objective evidence, that specified requirements have been fulfilled. Confirmation can comprise activities such as: performing alternative calculations; comparing a new design specification with a similar proven design specification; undertaking tests and demonstrations; and reviewing the document prior to issue.

Abbreviations

BHI: Brain Heart Infusion

CAP: College of American Pathologists

CDC: Centers for Disease Control and Prevention

CLSI: Clinical and Laboratory Standards Institute

CV: Coefficient of Variation

DTS: Dried Tube Specimen

DBS: Dried Blood Spots

EDTA: Ethylenediaminetetraacetic acid

EIA: Enzyme Immuno Assay

ELISA: Enzyme-linked immunosorbent assay

EQA: External Quality Assessment

GLP: Good Laboratory Practice

GCP: Good Clinical Practice

HCW: Health Care Worker

HIV: Human Immunodeficiency Virus

ID: Identification

IFU: Instructions For Use

ISO: International Organization
for Standardization

MCH: Mother and Child Health

NPV: Negative Predictive Value

NRL: National Reference Laboratory

POC: Point-of-care

PPV: Positive Predictive Value

PMTCT: Prevention of Mother To
Child Transmission

PT: Proficiency Testing

PP: Proficiency Panel

QA: Quality Assurance

QC: Quality Control

QMS: Quality Management System

RDT: Rapid Diagnostic Test

RPR: Rapid Plasma Reagin

RT: Room Temperature

SESAI: Secretaria Especial de Saúde Indígena

SOP: Standard Operating Procedure

STI: Sexually Transmitted Infection

TPHA: *Treponema Pallidum* Particle
Agglutination Assay

TPPA: *Treponema Pallidum*
Haemagglutination Assay

UVRI: Ugandan Virus Research Institute

VCT: Voluntary Counselling and Testing

WHO: World Health Organisation

Preface

Serologic tests to detect the presence of antibodies to syphilis in individuals play an increasingly important role in efforts to address the global epidemic of the disease.

With the exponential growth of programmes for prevention of mother to child transmission of HIV, and with increasing emphasis both on prevention of HIV infection among persons with conventional sexually transmitted infections and on blood safety, these tests will be an essential tool for the diagnosis of a disease which has largely been neglected. Current technologies available for syphilis testing include the standard laboratory-based tests such as the rapid plasma reagin test or enzyme immunoassay assay technology. In many situations, however, rapid tests for syphilis will be the most efficient and perhaps the only feasible way to provide information about syphilis status.

The accuracy and reliability of diagnostic/ laboratory testing will be critical to the success of syphilis elimination programmes. In order to ensure this reliability and reduce errors to a minimum, a quality system that addresses all aspects of testing is essential. The quality system is important in any laboratory or testing site and applies to all testing and lab based activities, including simple-to-perform tests. It is also essential to set up all the elements of a quality system in sites conducting only rapid syphilis testing.

The simple rapid syphilis tests discussed in this document are single use, disposable devices that may be used to directly test whole blood specimens, serum, or plasma. Although rapid syphilis tests are simple to use and can provide reliable results when the manufacturer's directions are followed, errors can occur at any point in the testing process. These single use devices present unique challenges:

- Testing is often performed by persons without formal laboratory training.
- There may be no residual specimen that can be checked or re-tested.

- Conventional quality control methods cannot be used.
- There are particular problems associated with efforts to provide conventional proficiency testing.

Because of this, and to reduce any errors during testing, the test site must have a quality assurance programme in place. This programme must take into account all levels of healthcare facilities, from the large clinic or hospital where on-site laboratory support is available to outreach settings with fewer personnel and resources.

The purpose of this document is to establish guidelines for applying quality system essentials to syphilis rapid testing. It is intended to provide assistance to all persons involved in the planning and implementation of rapid syphilis testing. The document should be useful to government health officials, those responsible for Sexually Transmitted Infection and Mother and Child Health programmes, and those responsible for managing HIV voluntary testing and counselling sites. It also provides information that will be useful for testing personnel, both trained laboratory technologists and those with no laboratory training.

As syphilis rapid testing is initiated and/or expanded into large numbers of testing sites, it will be very important to implement these guidelines, including the essential monitoring processes, to assure quality and reliability of test results.

These guidelines have been designed in line with the Centers for Disease Control and Prevention guidelines for designing a quality system for HIV rapid testing programme ([Guidelines for Assuring the Accuracy and Reliability of HIV rapid testing: Applying a Quality System Approach](#)). In addition, the guidance provided in this document can be readily adapted for use for other rapid diagnostic test programmes.

Background

All laboratory testing, including rapid testing for syphilis, consists of a series of processes and procedures that must be carried out correctly in order to obtain accurate results.

An approach that monitors all parts of the testing system is needed to ensure the quality of the overall process, to detect and reduce errors, to improve consistency between testing sites, and help contain costs. This approach to laboratory quality is called a quality management system. The quality management system includes policies, quality assurance, quality control, and quality improvement. In this document, it is divided into the twelve essential elements described in the Clinical and Laboratory Standards Institute document HS1-A, "A Quality System Model for Health Care.

The performance of rapid syphilis testing presents special challenges when you are undertaking measures to improve test reliability and accuracy. You need to consider these challenges when developing a plan for the implementation of a quality management system.

In many instances, rapid syphilis testing will be conducted by health care workers who do not have specific laboratory experience or by lay counsellors with no formal health care training. The training programme for these non-laboratory staff members must provide all the necessary laboratory skills, including specimen collection and laboratory safety. Sufficient time for practical hands-on work, as well as some measure of competency on completion of the training, is very important.

“A quality management system, is defined as the organizational structure, resources, processes, and procedures needed to implement quality management at the laboratory or testing site.”



Dried Tube Specimen Testing, Peru

1. A Quality Management System Approach for Rapid Diagnostic Tests

What are the objectives of a Quality Management System for Rapid Syphilis Tests?

- To assess the quality of specimen/sample collection and processing
- To document the validity of the test methods
- To monitor reagents, equipment, and the performance of test procedures and personnel
- To review test results
- To provide feedback for corrective action

Table 1. Principal components of a Quality Systems Programme

What is a Quality Management System?	What is Quality Assurance?	What is Quality Control?
The organizational structure, resources, processes, and procedures needed to implement quality management at the laboratory or testing site.	An integrated management function that deals with setting policy and running an administrative system of controls to ensure the usability of the product, and ensures that a process or device is of the quality needed and expected by the operator.	A system of routine standard technical activities to measure and control the quality of testing against a defined set of criteria or standard, ensuring correct operation of the rapid diagnostic tests and correct diagnosis.

Table 2 provides an overview of the key activities and responsibilities of a Quality Management System for Rapid Diagnostic Tests.

The expected outcomes of a successful quality assurance programme include:

- Standardized process of testing for patient diagnosis.
- Improved quality of diagnosis and quality of care.
- Empowerment of health care workers and enhancing the skills and motivation of health care workers.

Table 2. Principal components of a Quality Systems programme:

Activity	Quality Control Process	Applicable to
Documents and Records	Document Control Audit/supervisory visit/on-site monitoring	Health care workers Lab personnel District supervisors/Monitors Quality Officers
Ensuring biosafety measures are in place	Audit/supervisory visit/ on-site monitoring	Health care workers Lab personnel District supervisors/Monitors
Ensuring all health care workers and lab personnel have received adequate training and re-training when necessary	Audit/supervisory visit/ on-site monitoring External Quality Assessment	Health care workers District supervisor/ Programme coordinator
Ensuring a stock management system is in place	Audit/ supervisory visit/ on-site monitoring	Health care workers Lab personnel District supervisors/Monitors
Ensuring correct methods of specimen collection	Audit/ supervisory visit/ on-site monitoring	Health care workers District supervisors/Monitors
Ensuring quality of reagents used	Incoming Inspection Internal quality control External Quality Assessment	Health care workers Lab Personnel
Performing the tests with proper precision and accuracy	Internal quality control External Quality Assessment	Health care workers Lab Personnel
Interpreting the results correctly	External Quality Assessment Confirmatory re-testing	Health care workers Lab Personnel
Monitoring and evaluation, Coordinating and supervising	Audit/ supervisory visit/ on-site monitoring	District supervisor/ Programme coordinator
Giving timely feedback	Audit/ supervisory visit/ on-site monitoring	District supervisor/ Programme coordinator Lab personnel
Detecting errors in the testing process and taking corrective actions	Audit/ supervisory visit/ on-site monitoring	Health care worker Lab personnel District supervisor/ Programme coordinator

2. Documents and Records

Standardized documents and records should be developed at the national level in order to assure conformity to national standards and for ease in collecting national data. Documents and records must be maintained in such a way that they are always up-to-date and accurate, readily accessible by laboratory staff, and protected from damage and deterioration. Retention times for documents and records should be established. Policies should be developed to ensure confidentiality when appropriate.

2.1 Documents

2.1.1 Definition

Documents are written policies, process descriptions and procedures, and any blank forms used in the testing process. Documents developed within the quality system include the written standard operating procedures, safety policies, personnel policies, and all standard blank forms, such as reporting forms.

External documents will also be used and can provide important information. Examples of external documents used in a syphilis rapid testing site include information from the kit manufacturer, references from journals, and any service manuals such as for centrifuges or refrigerators.

2.1.2 Management of documents

Documents should be consistent with national policy, to assure uniformity and adequacy of data. All documents need to be managed with a tracking system, to ensure that all testing sites have current information on hand and that outdated documents are archived and ultimately discarded to avoid confusion at the worksite. All documents and records should have a standardized numbering system with version numbers and dates of update.

Brazil

In Brazil, syphilis and HIV rapid testing is carried out on a laminated A3 worksheet. The sheet is divided in two: the HIV test is placed on one half and the syphilis test on the other. This helps health care workers to avoid mixing up the tests, which look very similar. The worksheet also summarises the test procedure.

A testing and treatment algorithm for both syphilis and HIV is given on the reverse side of the worksheet.

Refer to [Appendix 12](#) for an example of this worksheet. A pictorial worksheet is also provided. It details the steps for reconstitution and testing of Dried Tube Specimens. Refer to [Appendix 13](#) for an example.

2.2 Records

2.2.1 Definition

Records result from carrying out processes and procedures within the testing process. Examples include:

- Worksheets.
- Test result reports.
- Labels.
- Temperature and maintenance charts.
- Quality control results and charts.
- External quality assessment activities with results and corrective action.
- Inventory lists.

The records include everything used to capture information, activities, or results when performing a procedure. They may be kept on paper, or electronically using a computer system. Records allow for the continuous monitoring of the quality system.

2.2.2 Management of records

Records for syphilis testing sites should be standardized and distributed on a national level. At a minimum, worksheets should include:

- Space for the date and time.
- Client identifiers.
- Name of the person performing the test.
- Name and lot number of the kit used.
- Quality control results.

A separate quality control chart should be maintained to allow for analysis and quick review of quality control results. Personnel records on training, competency evaluation, and work injury should be kept. All adverse occurrences, including any corrective action taken, should be recorded.

An example list of records that can be kept at central and site labs for rapid syphilis test programmes:

- Characterization of dried tube specimens (DTS) using rapid plasma regain / *Treponema Pallidum* Haemagglutination assay.
- Proficiency testing results form.
- Quality control log (internal quality control, external quality assessment).
- Quality control failure log.
- Training log.
- Accident Report form.
- Incoming Inspection form.
- Quality assurance checklist for site monitoring visits.



Documentation system at health centre, Lima, Peru

3. Organization and Management: Structural Set-up of Quality Assurance

Strong commitment from top-level managers is essential to the success of the overall quality programme. This commitment is important at all levels, and national laboratory leaders will need both to provide strong leadership from the national level, and to motivate and help laboratory managers throughout the country to understand the system and commit to its success.

3.1 Responsibilities at the national level

3.1.1 Establishing a laboratory quality system

The implementation of a quality system requires commitment from the top levels of management. The Ministry of Health, including national laboratory leaders with appropriate government authority, should establish a national quality system. This should include:

- A national office of quality assurance or quality management.
- The identification of a national quality officer or manager.
- The identification of a multisectoral working team, in order to extend the quality system to all aspects of testing practices and to avoid vertical decisions and assessments.
- Extend the quality system to all tiers (central, regional, district, point-of-service) of the laboratory network, and the involvement of all service providers at all levels.
- Extend the quality system to all laboratory testing, including serological testing for syphilis.

3.1.2 Planning for the management of rapid syphilis tests

An overall, country-wide plan for the management of syphilis testing, including the role of rapid syphilis tests, is essential. The following steps will be needed to establish this plan:

- National policies for the use of rapid syphilis tests must be established. Issues to be addressed include:
 - The use of rapid testing as an alternative to conventional laboratory testing for syphilis. When and where is this appropriate?
 - Personnel issues. Who will be allowed to perform syphilis rapid testing, and what training and certification will be required? How will appropriate supervision be provided?
 - Legal requirements that might apply to testing. Examples include country requirements for existing personnel certification as well as existing national laboratory and safety standards.
 - Evaluation of the test kits that will be used in the country and establishing an algorithm to be used for testing.
 - Linkages between conventional serological tests for syphilis and rapid tests.

- Development of standard operating procedures to be used in all testing sites.
- Confidentiality issues.
- Requirement for corrective or remedial action.
- A strategic plan for implementation of syphilis rapid testing should be developed. This plan will include provision for training of personnel and for continuous monitoring and improvement of the testing process. It is important to establish a timeline as well as processes to deal with many elements of the quality system.

Monitoring processes should be established to identify problems and confirm that the system is working. There must be a plan for solutions to the problems, and records kept of corrective actions taken. [Section 8.4.4](#) of this document and [Implementation 4](#) of the Toolkit provide more information in implementing a monitoring plan for the quality system.

3.2 Responsibilities at the site where testing occurs

Provisions must be made at the laboratory or point-of-care facility (such as an antenatal or Sexually Transmitted Infections clinic, or a voluntary counselling and testing centre) for oversight of testing, to ensure that the necessary staff and supplies are available, and to ensure that confidential records are established and maintained. There are several steps in this process.

- Management of the syphilis testing programme at each site must be assigned to one person; this person may be designated as a quality officer. The responsibility should be assigned to someone with authority to make and implement decisions, who has strong knowledge of syphilis testing procedures and a complete understanding of the essentials of the quality system. In some settings, one quality officer might serve several sites. The quality officer should have a clear channel of communication to the Ministry of Health or policy body, as well as to all staff who perform testing, so that any changes in procedure or other important information can be shared in a timely fashion.
- Standard operating procedures must be established at each site. The step-by-step set of instructions that outlines all the processes for conducting testing must be accessible to everyone who performs tests.
- Local management must ensure that all testing is performed by staff who have been trained and certified according to national requirements. The quality officer must also have a plan for evaluating staff, initially and on an ongoing basis. If there is no national certification programme, local management must ensure that staff are trained and competent to perform rapid syphilis testing according to national guidelines.
- Oversight of the recordkeeping system must be provided.
- Finally, the quality officer must ensure that all other components of the quality system are in place before testing is begun at a site. No testing should be conducted until the site can be demonstrated to be properly prepared.

4. Facilities and Safety

4.1 Facilities

Every site where syphilis rapid testing is performed must have a physical space that is appropriate for the testing. This should include:

- An adequate working surface that can be easily cleaned and maintained
- Assurance of an environmental temperature that does not exceed that required by the testing kit
- Refrigeration if needed
- Facilities for hand washing and cleaning.

4.2 Safety

Procedures to handle biohazardous material safely must be made clear and staff must follow them. These will include:

- Instructions on use of gloves, use of closed footwear, hand washing, handling and disposal of sharps, spill containment and disinfection.
- Basic safety procedures that are clearly visible in the workspace.
- General policies such as “no eating, drinking, or smoking” or “no unauthorized persons in the testing area”, which must be enforced.
- Procedures for the safe disposal of all specimens and materials used in testing, which must be observed at each site. This is essential in order to protect workers performing the tests as well as any others who might be exposed to discarded materials. All specimens and materials must be handled as if capable of transmitting an infectious disease. A procedure for workers to follow if there is accidental exposure of staff to biohazardous material. This procedure, as well as a list of persons to contact in an emergency, must be readily accessible to all facility staff. It is recommended that all persons performing rapid syphilis tests should know their serostatus for both syphilis and HIV.
- A procedure for workers to follow if there is accidental exposure of staff to biohazardous material. This procedure, as well as a list of persons to contact in an emergency, must be readily accessible to all facility staff. It is recommended that all persons performing rapid syphilis tests should know their serostatus for both syphilis and HIV.

Full safety requirements for testing blood/serum specimens are very detailed. A complete set of country guidelines should be made available at any site where testing is performed. Useful references include: International Organization for Standardization (ISO), World Health Organization WHO Biosafety guidelines, CDC Biosafety Guidelines.



Biohazard precaution measures

5. Personnel and Training

A standardized training programme for laboratory-based and non-laboratory staff should be developed and implemented at all levels of service delivery. Frequently, the training of large numbers of staff will be accomplished at the time when testing is widely implemented: at the same time, for example, as the inclusion of a new group of antenatal clinics or the expansion of syphilis testing in Preventing Mother to Child Transmission programmes.

The national training plan must make provision for the training of new staff as they are hired and added to the workforce. This is critical to the maintenance of quality, reliable testing. It may at times be a challenge, as training sessions may have to be done for just one or two persons. Nonetheless, all new staff should undertake the same training programme that is used for initial training

Detailed guidance for training staff on the use of rapid syphilis testing, including quality assurance and quality control aspects is given in [Implementation 3](#) of this Toolkit.



Training workshop for laboratory workers in Lima, Peru

6. Equipment

One of the great advantages of using rapid, simple technology for syphilis testing is that **little or no equipment is required**. However, in some settings the use of whole blood or serum may require a centrifuge and pipetting devices. In this case, a plan for calibration and maintenance should be developed.

There may also be a need for refrigeration to store syphilis rapid test reagents or specimens; if so, temperature checks with documentation and a maintenance plan must be effected.



Rapid syphilis testing in primary health centre in Tanzania

7. Purchasing and Inventory

It is essential that dependable and reliable test kits and supplies are available. This requires a national plan for procurement and distribution, as well as careful management of supplies and reagents at the testing site.

7.1 Responsibilities at the national level

Most countries use a tender process for the procurement of reagents and supplies for all laboratories and testing sites managed through the Ministry of Health. It is important that supplies and reagents be carefully selected and that they are ordered in sufficient quantity to last until the next tender.

The kits purchased must have an expiration date that is far enough into the future to allow for efficient use and to prevent waste. A policy of “first expired, first out” will also help to assure minimum waste.

The Ministry of Health/ national reference laboratory must have some means of assessing the quality of the kits, reagents, and supplies as they are received by the central purchasing body, to ensure that standards and specifications are met. It is recommended that each lot number be checked by the national reference laboratory before distribution.

It will be necessary to implement a distribution plan that allows these reagents and supplies to reach all testing sites within the appropriate time frame and prior to expiry. The plan must take into account emergency or unexpected needs.

7.2 Responsibilities at the testing site

An inventory record for kits and supplies should be maintained. Each site should determine re-order levels for each item in the inventory based on workload and usage. This will allow for ordering in a timely manner, so that the testing site always has the necessary reagents and supplies and no interruption in testing will occur. A method for calculation may be found in [Management 2](#) of this Toolkit.

On receipt of new supplies and reagents, the inventory record should be updated and all of the new material stored under the appropriate environmental conditions. [Management 2](#) of this Toolkit provides examples of forms that can be used for inventory records.

To avoid waste, sites should follow the principle of “first expired, first out”. The kits that expire earliest must be used first.

Further guidance on managing and maintaining stock at the health centre is provided in [Management 2](#) of this Toolkit.

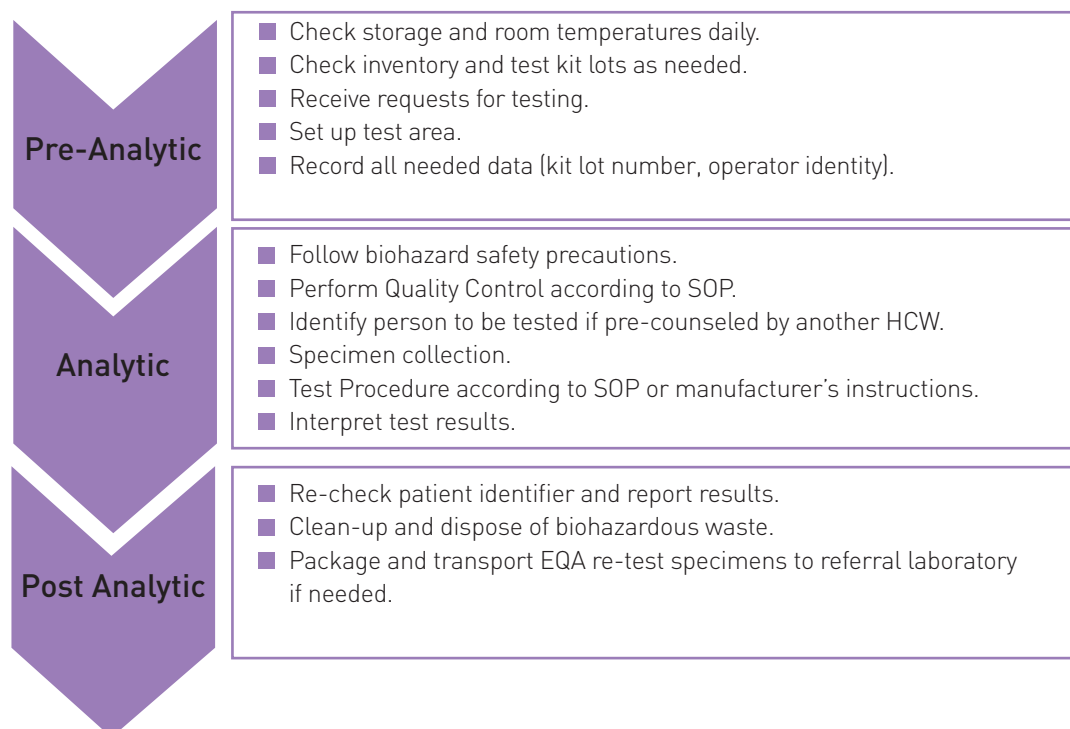
8. Quality Control

Quality control refers to activities and techniques carried out to ensure that testing procedures are performed correctly, that the environment is suitable for reliable testing, and the test kit works as expected to produce accurate and reliable results.

Quality control usually includes testing specimens of a known value using the same reagents and equipment that are used for the specimens being measured. Since rapid syphilis test kits are single use devices, this approach is not possible. Quality control specimens must therefore be used in a manner that monitors the correct performance of the test by the operator and the capacity of the test kits to work properly. While it is not possible to test each kit, quality control specimens can be used to detect damage of an entire batch or lot number of kits, whether it is caused by improper storage or handling or through manufacturing defects.

The quality control testing process follows the path of workflow. The path of workflow is frequently described as the steps done before testing (pre-analytic), those done while testing (analytic), and the steps that follow testing (post-analytic). When using rapid syphilis test kits, there are a number of steps in these three parts of the path of workflow that are essential in order to assure accurate and reliable test results. Some of these activities are detailed in Figure 1.

Figure 1. Process Control Activities



A standard operating procedure (SOP) must be developed to provide detailed instructions on all aspects of the testing. It should include:

- Transport of specimens.
- Storage and inventory information.
- Test requisition.
- Environmental requirements.
- Specimen collection and management.
- Test performance.
- Quality control instructions.
- Test interpretation.
- The reporting and recording of results.
- Appropriate use of the testing algorithm.
- Any external quality assessment requirements.

Each test product will need its own standard operating procedure. A written standard operating procedure should be available at each testing site, and should always be followed when conducting tests. A chart showing a simplified version of the procedural steps (work instructions) is very useful and should be provided at the point of testing performance. The test site must have written instructions on all policies and procedures to be followed when conducting tests, including personnel training and certification requirements, competency checks, confidentiality policies and safety.

See an example of a Standard Operating Procedure for Manufacture of Dried Tube Specimens in [Appendix 4](#).

8.1 Incoming Inspection of rapid syphilis test kits

Incoming inspection testing is carried out on each new lot or shipment of rapid test kits arriving in-country from the supplier. It ensures the validity and integrity of test kits and lots after shipping. A representative number of test kits are tested using a known negative or known positive control at the central or reference laboratory. Each new lot of tests received should be tested in parallel with the old batch to confirm lot to lot agreement.

8.2 In-built procedural control

Many rapid diagnostic tests have in-built procedural controls in the form of a test control line. In some kits, these controls may be provided as a separate material, but will still be used with each test. The in-built control verifies that the specimen was adequate and that the complex of specimen and reagent flowed through the device as intended. It does not validate the testing process or the tester. The operator should follow manufacturer's instructions and explanation of the location and functioning of the in-built procedural control.

Zambia

In Zambia, rapid syphilis test kits are validated to confirm that they are in good working order upon receipt at the central laboratory from the supplier. This is done by testing a random selection of 1% of the kits in each batch of test kits using known syphilis-positive and syphilis-negative specimens. The known syphilis-positive and -negative samples are provided and characterized by College of American Pathologists (CAP) surveys.

The positive samples are tested on rapid plasma reagin, titrated and confirmed on *Treponema Pallidum* Haemagglutination technique. The central lab is registered with the College of American Pathologists and receives syphilis proficiency panels 3 times a year. This incoming inspection process confirms the validity of the test kits prior to their distribution to the health centres.

8.3 Internal Quality Control

Internal quality control evaluates the accuracy of the test and verifies the operator's ability to perform the test and interpret the test result correctly. It ensures reliability of the test result on the day of testing. Internal quality control should include the testing of at least one known syphilis-positive and one known syphilis-negative specimen. If possible, a weakly positive syphilis specimen should also be included.

The Ministry of Health should establish policy for how and when internal quality control material should be used. This information must be provided and described in the standard operating procedure.

The frequency of use of quality control material is dependent on several factors. The condition of the kits must be evaluated over time. It will be important to check kits fairly often in areas where environmental conditions can be extreme and difficult to control, and where transportation can be challenging. When running controls for rapid syphilis testing, it is important to use both a negative and a positive control. Whenever possible, a weakly reactive positive control that has been validated to yield weakly reactive results on all rapid test kits used should be included.

8.3.1 Source of quality control materials

Dried Tube Specimen

Dried tube specimens [DTS] can be used as an alternative to dried blood spots (DBS) for internal quality control and/or proficiency testing. Dried tube specimens are a dried form of serum or plasma with known serostatus. They are prepared using a standardized protocol (given in [Appendix 4](#)) and characterized using the reference standard available (e.g. rapid plasma reagin, *Treponema Pallidum* Particle Agglutination assay/ *Treponema Pallidum* Haemagglutination assay). Dried tube specimens are cold-chain-independent and can be shipped to and stored at health centres in non-refrigerated conditions.



Internal QC pack using DTS in Peru

The advantages of using dried tube specimens include the following:

- Dried tube specimens are stable at elevated temperatures for at least 3 - 4 weeks.
- They are cold-chain-independent and can be easily transported by mail or vehicle.
- A Dried Tube Specimen Panel can be made in-country, maintaining low cost when specimens are already available and allowing the quality of testing to be continually monitored.
- They can be used for Proficiency Testing and/or as Internal Quality Control materials.

The Dried Tube Specimen Panel used to evaluate the health centre or laboratory's performance **MUST NOT** be treated differently from any patient specimens tested.

It is recommended that quality control materials be distributed with the test kits. This will assure that each site has sufficient quality control material for use with the kits.

8.3.2 Troubleshooting and corrective actions when internal quality control results are out of range

When an internal quality control does not give results as expected and are out of range, the laboratory or clinic supervisor should be notified. S/he will decide how the non-conforming test result should be investigated. Errors in results may occur for a variety of reasons. Operator error in test performance is one cause of discrepancy: this will require additional quality assurance and training at the site. A common source of error is a transcription mistake at some point in the process.

Errors may be produced if the dried tube specimens were inadequately reconstituted at the health centre/laboratory or there was an error in manufacturing at the central lab. In addition, errors may be produced if the test kits are improperly stored and/or transported.

Any patient test results that have been generated since the last controls were run should be considered invalid until troubleshooting is undertaken to determine the source of the problem.

[Figure 2](#) details a process flow for corrective action when an internal quality control is out of range. Refer to [Section 10](#) of this document for further information on managing errors and problems in the test process.

Peru

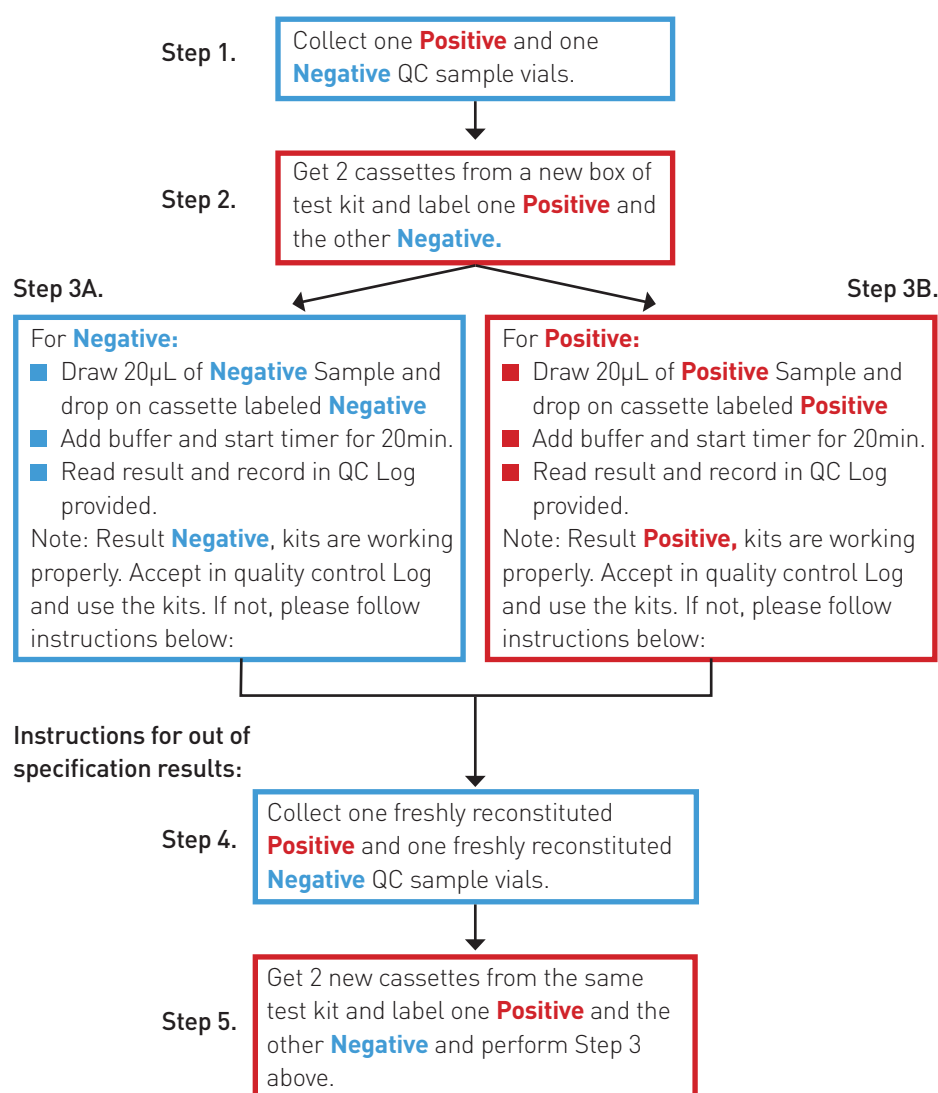
At the time of press, a real-time stability study of rapid syphilis tests was being undertaken in the various climatic settings within Peru. These include: the Iquitos Jungle (high temperatures and high humidity); the Piura Coast (dry with high temperatures); and the Cerro de Pasco Andes (high altitude, very cold and dry). Test kits in the various settings were compared to control kits stored in the reference laboratory in Lima at 4 °C (cold room/refrigerator) and 37°C (incubator) and 15 – 27 °C (storage room). At the time of this publication, 6 months of real-time study demonstrated equivocal performance of test kits with control kits.

For further information on this stability study, refer to <http://www.proyectocisne.org/> where full results will be published.



Temperature monitoring at health centre in Lima, Peru

Figure 2 Corrective Action Testing Algorithm when Quality Controls are out of range



8.3.3 Record-keeping

A standardized method for record keeping should be provided by the national reference laboratory or central laboratory. This should include a standard worksheet for recording quality control data and a corrective action algorithm for troubleshooting an out of specification quality control result.

An example of a Quality Control Record Form, including logging of corrective actions, is given in [Appendix 7](#).

8.4 External Quality Assessment

Through external quality assessment, the performance of a testing site can be independently evaluated from outside the laboratory or testing site. Methods for external quality assessment include traditional proficiency testing, re-testing of specimens, and careful on-site monitoring using a checklist and knowledgeable assessors.

8.4.1 Proficiency testing using a Proficiency Panel

Traditional proficiency testing is organized and conducted by a reference laboratory or centre. At regular intervals, a panel of specimens of known reactivity is sent to all participants, who test the specimens and return results to the reference laboratory. The data is analyzed and information is sent back to the participating testing sites.

Proficiency Testing can be performed using any of the following specimens:

- Liquid serum or plasma specimens
- Freeze-dried specimens
- Dried tube specimens

The disadvantages of using liquid serum or plasma specimens for an external quality assessment programme are the limitations in shipping and storage: they require cold storage and transportation; there is an increased risk of bio-hazard spill; it is costly; and it is impractical for remote sites.



Reconstitution of DTS



Proficiency Panel results using rapid syphilis tests in Brazil

The number of correctly identified specimens/total number of vials) x 100% = score. (A cut-off percentage or passing grade should be determined as good practice by the central, or coordinating, laboratory.)

Brazil

Dried tube specimens are used for proficiency testing among health posts in the Amazonas region of Brazil. The cold-chain-independency of the dried tube specimen makes it an ideal form of specimen to be shipped over long distances with unreliable modes of transport and with high temperatures and humidity. The frequency of dried tube specimen testing as part of an external quality assessment programme for rapid syphilis testing is largely dependent on the accessibility of the clinics/health posts. Distribution is mainly opportunistic: dried tube specimens are sent for testing whenever a Secretaria Especial de Saude Indigena (SESAI) technical coordinator is making a routine visit to the health posts.

Follow-up on results can be conducted that day or by cell phone or by mail if testing is not performed on the same day.



Rapid syphilis and HIV testing in the Ianomami community of Amazonas, Brazil

8.4.2 Scoring and follow-up of Proficiency Panel test results

The district supervisor or lab coordinator will collect all proficiency testing record forms from participating laboratories. Results should be entered in an excel spreadsheet. Proficiency panels should be scored and the final score converted to percentages using the following equation shown left. The lab or district supervisor should follow up with those testers or facilities that obtained less than the cut-off percentage or passing grade and undertake the necessary corrective actions or re-training to resolve any issues.

Zambia

The management team for the rapid syphilis testing programme in Mongu and Lusaka in Zambia have implemented a corrective action plan for health care workers who obtain a final score of less than 67% on external quality assessment. All individuals or facilities that scored less than 67% overall on the Dried Tube Specimen Proficiency Panel are asked to test a second proficiency panel as a form of re-training.

Before testing the second panel, the internal monitors or supervisors will visit the facilities and observe and provide guidance as the health care workers re-test the original proficiency panel so that any problems with the weak positive samples can be addressed. The second proficiency panel will then be distributed to the facilities, where health care workers will test unaided.

Proficiency testing for rapid syphilis tests has some limitations. The panel of specimens sent to the testing site will not necessarily be tested by all staff, so this is not a good measure of individual performance. The sample size is small, so the ability to detect errors is impaired. Furthermore, preparing and distributing specimens for proficiency testing may be burdensome for national referencelaboratories. Proficiency testing is provided in some locations, and when available it is a useful tool in combination with on-site monitoring.



DTS Panel Pack used in Peru

In the 7 study countries where a quality system approach to rapid syphilis testing was introduced, health care workers were highly motivated by the rapid testing and felt empowered by the capacity to perform patient diagnosis at the point of care and the associated responsibility. It was widely acknowledged that the quality controls built confidence around the correct operation of the test and the correct diagnosis of the patient.

SOPs for preparation and testing of DBS are available at: <http://www.lshtm.ac.uk/itd/crd/research/rapid-syphilis-toolkit/index.html>

Haiti

In Haiti, a panel of 5 dried tube specimens of known status are prepared and sent by the central lab to the different sites for testing and reporting on a quarterly basis. These specimens are typically made up of 2 negative, 2 weakly positive and 1 strongly positive samples.

The passing grade for a round of testing is 80%. Labs that have a grade of 80% or less on any event more than once per year must be considered for personnel retraining. The sites must keep a record of the proficiency testing and evaluation results. The sites must also provide documented evidence of a review of evaluation results and corrective actions in the case of an evaluation result of less than 100%.

Uganda

The Ugandan Virus Research Institute (UVRI) is responsible for the manufacture of dried tube specimens for proficiency testing and for the review and follow-up of completed results from the facilities for the rapid HIV and syphilis testing programmes.

The manufacture of dried tube specimens for rapid syphilis testing is integrated with that for HIV rapid syphilis testing. The panels currently remain separate but the scheduling for manufacture, distribution to facilities and follow-up and review is coordinated for both.

Brazil

To ensure that multiple operators at a health facility never test the same proficiency samples and to avoid sharing of test results, the research team in Brazil prepared different lots of dried tube specimen panels for the syphilis and HIV proficiency panels.

4 separate panels with individual lot numbers were created with 6 panel members each by selecting different panel members for each panel lot, but always ensuring that each panel lot has at least one strong positive, one weak positive and one negative sample.

8.4.3 Re-testing of specimens

With this external quality assessment technique, serum is collected from the client at the time of rapid testing. The serum is tested using a treponemal syphilis test such as a *Treponema Pallidum* Particle Agglutination assay or Enzyme Immuno Assay (EIA), and the results of this test, or "re-test," are compared with that obtained from the final syphilis rapid test result. A common model in use is the re-testing of 5% - 10% of rapid test specimens, randomly selected.

Re-testing with Dried Blood Spots

With dried blood spots, drops of blood are collected onto a filter paper at the time of testing. Once the drops are dried, the filter paper cards with the dried blood spots can be sent to the reference laboratory for retesting with a treponemal based syphilis assay. No special storage conditions are required for DBS. The benefits of dried blood spots are that they easily collected and correct collection and storage of samples requires minimal training. For temporary storage, humidity should be controlled by placing the DBS in plastic sealable bags with desiccants. After retesting, laboratory results should be entered in Excel or comparable software to review the performance of the health centres over time. The district supervisor or lab coordinator should follow up and provide corrective actions with those health centres that perform below a determined percentage cut-off level. Dried blood spots for syphilis can also be integrated with the HIV or malaria quality control system to minimise sample collection, transport and effort. The quality control of syphilis, malaria and HIV testing can be combined by testing one filter paper for the three different pathogens.

Tanzania

Dried blood spots have been used over a 4 month period for re-testing of patient samples for syphilis and HIV as a quality control method in the Geita district of Mwanza, Tanzania. The study was coordinated by the National Institute for Medical Research, Mwanza in Tanzania. This quality control method was successful in identifying those clinics that were performing well and clinics that would require additional support and/or re-training by district supervisors if they performed below the determined cut-off performance level. The method was tested

in a hospital setting, as well as at different health centres and dispensaries. Protocols, guidelines, excel and Epi-info templates used during the study can be found at the website <http://www.lshtm.ac.uk/itd/crd/research/rapid-syphilis-toolkit/> in a zip file. The excel file (DBS results analysis.xls) was useful for visualising the performance of the different clinics. The excel file generated a cut-off line of the median \pm 2 standard deviation. If a clinic performed 2 months below this cut-off, a supervisory visit was recommended to perform any corrective actions, such as troubleshooting, or re-training on site.

Re-testing of specimens has limitations. In many countries there is lack of capacity at the national reference laboratory for re-testing the large number of samples and for conducting the needed analysis of data. Long delays in completing the re-testing results in delayed identification of problems. An outline of the operational issues that must be considered before a re-testing programme is implemented is given in [Appendix 8](#) and [Appendix 9](#). Finally, statistical analysis reveals that for low-volume sites, a very large percentage of samples would have to be re-tested in order to detect errors.

The table below summarizes the statistical information. (Note: The number of specimens tested is independent of time.)

Table 2. Re-Test Size (and percentage) needed to provide 95% confidence of detecting at least one discrepant result, when the underlying error rate is 1% or 5%E

Volume (per site)	1%* error	5%* error	Re-testing feasibility
Very low 50 specimens	Re-test 48 specimens (96%)	Re-test 31 specimens (62%)	No
Low 500 specimens	Re-test 225 specimens (45%)	Re-test 56 specimens (11%)	Possible
High 5000 specimens	Re-test 290 specimens (5.8%)	Re-test 59 specimens (1.2%)	Yes

* 95% confidence

A full discussion of the statistical models is presented in [Appendix 9](#).

Note that these statistical models show analysis of site-specific data. Aggregate data could be obtained by combining data from several or many sites, and could be of some use in looking at the overall programme. However, aggregate data is of no value when evaluating site errors or taking corrective action.

It is recommended that:

- Re-testing be used to establish the competence of new staff or a new testing site.
- If on-going re-testing is performed, it must be based upon statistical considerations and a recognition that it will only be feasible in high-volume test sites.
- The outcome of re-testing must be analyzed for effective and timely feedback
 - in order to determine cost-effectiveness
 - to determine if corrective action can be taken if problems are identified
- If errors are not found as a result of re-testing, established sites should consider discontinuing re-testing.

8.4.4 On-site monitoring

External quality assessment can be accomplished by a careful on-site observation of the testing processes and procedures by a knowledgeable person or team. A checklist that allows for assessment of all parts of the quality system is an important tool for such visits. Refer to [Implementation 4](#) of this Toolkit for further information on implementing and conducting on-site monitoring, including a sample checklist.

It is recommended that:

- Major emphasis is placed on on-site monitoring in the external quality assessment plan. In low-volume sites this may be the only external quality assessment tool that is used.
- On-site monitoring should include all aspects of the quality system, including personnel competency and training, equipment policies, inventory control, quality control practices, records and documents, and facilities and safety.
- If other testing, such as HIV rapid testing, is performed at a rapid syphilis testing site, an integrated approach to on-site visits should be taken to assess all aspects of testing practices.
- The site visit should include observation of testing with specimens of known reactivity (proficiency panels).
- When possible, direct observation of interactions with clients is useful. Other means of assessing performance of testing personnel could include exit interviews with clients and use of “mystery clients” (persons with known syphilis serostatus who present anonymously).
- A standard checklist must be used for all visits.
- The on-site assessment should occur at least twice yearly at established sites, and at least quarterly for new sites or sites with new personnel. The frequency of assessments should be based on initial findings and the need for corrective action.
- The on-site visits should be instructional and provide a mentoring experience. The experience should not be punitive.

A plan must be established for corrective action related to findings during the on-site visit. All problems should be discussed immediately with on-site staff, and any needed follow-up activities including training should be undertaken in a timely manner.

Table 3. Key characteristics of Quality Control Events

Defining Characteristics	Incoming Inspection	In-built procedural control	Internal quality control	External Quality Assessment
Objective	Ensures validity and integrity of all new lots and shipments of kits and consistency of new lot against a previously used lot.	An in-built control that verifies that the specimen was adequate and that the complex (solution) flowed through the device as intended. Does not validate the entire testing process	Evaluates the accuracy of the test and verifies operator's ability to perform the test and interpret the test result. It ensures reliability of test result on day of testing.	Proficiency Testing Ensures inter-laboratory comparability and assesses proficiency of participating lab/clinic and individual tester
Responsible personnel	Central/Reference laboratory	Health care worker Lab Personnel	Health care worker Lab Personnel	Health care worker Lab Personnel
Frequency	Every lot/Every shipment	Every test	<ul style="list-style-type: none"> At least one/week preferably at beginning of week New operator/turnover of testing personnel New lot of kits New shipment of kits When environmental conditions are outside recommended range for stability as defined by manufacturer Large volume of testing/anonymous testing should run controls more frequently Troubleshooting when in-built procedural control invalidates the test result 	Periodic (monthly to quarterly)
Sample format	Serum or Dried Tube Specimen Known positive and known negative specimen	Whole blood, serum or Dried Tube Specimen	Serum or Dried Tube Specimen Known positive and known negative specimen	Dried Tube Specimen Panel including negative, strong positive and weak positive specimens
Storage requirements	2 - 8°C for serum Room temperature for Dried Tube Specimen	2 - 8°C for serum Room temperature for Dried Tube Specimen	2 - 8°C for serum Room temperature for Dried Tube Specimen	Room temperature for Dried Tube Specimen
Cut-off/Out-of-range result	Any specimen not correctly identified requires further investigation of lot/shipment of kits	No visible control line invalidates test run	Overall results of < x % agreement will require internal investigation (x value should be determined by the overall quality management team, refer to Appendix 1 for examples from other countries)	Overall results of < x % agreement will require internal investigation (x value should be determined by the overall quality management team, refer to Appendix 1 for examples from other countries)

9. Information Management

Records may be kept either manually or using computer technology. Where computer systems are available, laboratories are afforded many useful tools for managing client data as well as quality control and external quality assessment information.

For example, a system for tracking serum specimens collected for external quality assurance purposes would make it much easier to manage this aspect of the quality system function. If there is country-wide networking, the potential to correlate an individual's clinical data and laboratory results country-wide is valuable.

When computerized information management systems are available:

- Processes to ensure accuracy and reliability of data, and to protect data from damage and loss, must be put in place.
- Privacy and confidentiality of data must be strictly observed.
- Staff will need training to develop competency in use of computer tools, including use of the specific laboratory system, as well as in word processing, use of spreadsheets, and databases.

Zambia

The SmartCare electronic health record system has been developed by the Zambian Ministry of Health in collaboration with the Centers for Disease Control and Prevention and many other implementing partners. It is a clinical management information system used at the facility and district level which includes details on HIV/AIDS treatment, TB care, Voluntary Counselling and Testing, and antenatal care among others. Dedicated personnel have a login and password for access. Every quarter, information is uploaded on a flash drive/central hub, compiled and analyzed. Each patient is issued with a Smart Card that stores an individual's health information and is allocated with the patient number.

This allows continuity of care between visits, health services and health facilities. Staff also have flash drives for lower-technology connectivity. The individual's health record is also stored on the health facility installation database for backup and generation of facility level and health management information system reports. The Zambian Ministry of Health has installed SmartCare in over 200 facilities (clinical and district/provincial/national levels), in all districts, and patient enrolment to the system was more than 200,000 in 2008.

10. Occurrence Management

Errors and problems occur in the most carefully conducted and monitored testing environments. The purpose of a quality system is to reduce and minimize errors in the total testing process. In order to meet this goal, each testing site should have a method to detect and resolve problems. It is important to understand root causes and to take corrective action.

The following steps should be followed when adverse incidents, errors, and problems occur:

- Investigate the error or problem to determine cause.
- Take action to address the cause of the problem. Corrective actions may result in changes in policy or procedures to help ensure that errors will not re-occur.
- Communicate appropriately with all those affected by the error or problem, for example, nursing staff, physicians, and/or clients.
- Keep a record of all circumstances related to the error or problem. Also keep a record of corrective action taken and any communications with affected persons. This information is useful for those monitoring the testing, for any internal audits, and for use if further enquiries from patients or physicians occur.
- The Quality Officer has the responsibility to ensure that this process is followed, including all appropriate corrective actions taken.

11. Assessment

The key to a successful quality system is continuous improvement. An essential component of this process is assessment. Formal assessments may be external, performed by persons outside the laboratory or testing site, or they may be conducted by staff at the site and be internally managed.

The regular performance of internal quality assessments and audits can yield a great deal of important information about how well the laboratory or testing site is following its quality policies and procedures. It can also help to identify problem areas. Information on the internal audit process is widely available, and the International Organization for Standardization describes an internal audit process that is useful in laboratories. Smaller testing sites could use a more informal process.

Key indicators that should be checked to assess the performance of individual facilities with respect to the quality systems programme include:

- Number of tests or external control materials that expired before use or occurrences of expired tests used for diagnostic or quality control purposes.
- Number of days that tests/ quality control materials were stored/used outside of temperature specs.
- Frequency of external quality control testing compared with test site procedure.
- Frequency of invalid/incorrect test results when performing external control testing or patient testing.
- Proportion of negative and preliminary positive patient results.
- Proportion of reactive rapid test results confirmed positive.
- Health care workers' ability to manage the corrective action (according to the local programme's algorithm) following an out of range result.
- How to document and verify corrective action
- Comparison of total number of reactive rapid test results with number of confirmed positive results. If resulting ratio of false-positive rapid test results suggests the test is not performing according to the manufacturer's specifications, quality assurance managers should:
 - Evaluate expiration dates and testing area.
 - Review records of external control testing.
 - Perform troubleshooting according to manufacturer's instructions.
 - Evaluate facility testing procedures and if necessary, modify quality assurance protocol or retrain staff. If necessary, inform manufacturer.

Refer to [Implementation 4](#) of this Toolkit for further information on monitoring the quality system.

12. Process Improvement

Process improvement is the action of revising a process based on information gathered and the identification and rectification of problems. As used in this model, process improvement involves identifying an area to study, collecting information, evaluating the information, and taking corrective action based on the findings. For example, a testing site might decide to study its turn-around time.

This would require collecting data for a period of time, analyzing the data, evaluating whether the turn-around time is sufficiently short, and if not, implementing some steps to shorten the time.

All these efforts should be the responsibility of the Quality Officer, who should manage all processes related to assessment and process improvement, and who should communicate results of all projects to both the site staff and to appropriate higher level management.

The advantages of process improvement include:

- Improved processes can raise morale among staff and patients and can further motivate staff in maintaining a high level of quality testing and diagnosis.
- It can set a benchmark of performance beyond which system improvements can be made continually.
- Available data can be used to identify current gaps that need to be addressed (evaluation of quality indicators)

13. Business Plan

Decentralization of testing creates challenges in the assurance of both the quality of tests and of testing. National laboratories can play an important role in the provision of quality assurance, quality control and supervision in remote settings if adequate training can be provided on the manufacture, validation and shipment of proficiency panels in freeze-dried form.

A generic business plan should be developed to allow national programmes to estimate the capital investments upfront and the recurring costs of making proficiency panels and maintaining a system of supervisory visits to remote settings to ensure quality testing using rapid syphilis tests. A sample outline of a business plan for a Quality Management System is provided on the next page.

Sample Outline of a Business Plan for a Quality Management System

1. What are your goals for a Quality Management System?

2. What is your Design Concept for a Quality Management System?

Include the following :

- The type of Quality Control events
 - Internal quality control
 - External Quality Assessment
 - Re-testing of specimens
 - Incoming Inspection
- Number of samples per quality control event
- Frequency of quality control events
- Documentation system and Document Control
- Can it be integrated into an existing quality programme for other diagnostics?

3. Management & Organization

- What Human Resource Requirements are needed? Make a list of all personnel required, including roles, responsibilities and time required for each:
 - Programme Management
 - Laboratory Personnel
 - Clinic level
 - Monitoring and supervision
- Do you need to develop contractual agreements with other institutions and departments (for Monitoring and Evaluation, Training, Manufacturing of Dried Tube Specimens)?
- Draw up an Organization Chart

4. What Operations requirements are needed?

- Location and facilities:
 - Equipment requirements
 - Technological tools
 - Printed materials
- Supply and distribution
- Key Suppliers
- Human Resource Plan

5. Based on all of the above, draw up a Budget. What level of funding do you require for the Quality Management System? What are the financial commitments and who are they from?

6. What are your Outputs for the Quality Management System?

- Performance analysis
- Corrective actions and/or troubleshooting

7. What are your Outcomes for the Quality Management System?

- Performance grade, individual
- Performance grade, facility

8. What are the Challenges or Risks facing a Quality Management System for your programme (e.g. High staff turnover, Regulatory changes) and list the Contingencies that can be put in place to overcome them.

14. Frequently Asked Questions on Quality Systems

What is a Quality Management System?	A quality management system typically includes the organizational structure, resources, processes, and procedures needed to implement quality management. These principles include categories such as Documents and Records, Organization, Personnel, Equipment, Purchasing and Inventory, Process Control, Information Management, Occurrence Management, External and Internal Assessments, Process Improvement, and Facilities and Safety.
What is Quality Assurance?	Quality Assurance encompasses all procedures and activities directed toward ensuring that a specified quality of product is achieved and maintained. In the testing environment, this includes monitoring: raw materials; supplies; instruments; procedures; sample collection, transport, storage and processing; recordkeeping; calibrating and maintenance of equipment; quality control; proficiency testing; training of personnel; and everything else involved in the production of the data reported.
What is Quality Control?	<p>Quality Control is a set of procedures undertaken in a laboratory or clinic for the continuous assessment of work performed. It includes the evaluation of tests to decide whether they are reliable enough for results to be released to the requesting health care provider and patient.</p> <p>Quality Control includes testing control materials; charting the results and analyzing them to identify sources of error; and evaluating and documenting any corrective action taken as a result of this analysis.</p>
What are the different quality control processes that I should undertake at the clinic as part of the quality system for rapid diagnostic testing?	<p>Internal Quality Control is the evaluation of test and operator performance that includes quality control samples for which the tester knows the expected measurement result.</p> <p>External Quality Assessment, or Proficiency Testing is the evaluation of test and operator performance that includes a sample for which the tester does not know the expected measurement result. The process evaluates the laboratory's or clinic's performance on the testing of samples of external origin for the purposes of determining adequacy of the laboratory's pre-test, test, and post-test activities.</p>
What are the possible outcomes of the quality control testing process?	<p>Test results are within range: Record results: no further action is required.</p> <p>Test results are outside range: Inform the lab/health centre supervisor, who should perform a corrective action to find the source of the problem. Refer to Section 8 of this document for advice on corrective actions and an example corrective action algorithm.</p>
If an internal control result is outside range, may I continue reporting patient test results?	No. All patient test results generated since the last controls were run are invalid until troubleshooting to determine the source of the problem has been undertaken. Every effort should be made to establish the root cause of the out of range test result, as it may be caused by unstable test reagents or operator errors that can impact on patient diagnosis.

What is proficiency testing?	Proficiency testing is an external quality assessment programme, in which multiple samples (within a proficiency panel) are periodically sent to members of a group of laboratories or clinics for analysis and/or identification. Each laboratory's or clinic's results are compared with those of other laboratories and clinics in the group and/or with an assigned value, and reported to the participating laboratory/clinic and others.
Why is proficiency testing important?	Proficiency testing allows for the internal monitor, lab director and programme manager to monitor healthcare worker performance. Proficiency testing helps to ensure that all health care workers are able to perform the rapid diagnostic test, interpret the results correctly, and can help identify problems with health care worker performance of the rapid diagnostic test.
What are Dried Tube Specimens?	Dried tube specimens are a dried form of serum or plasma with known sero status. Because they exist in powder form, they are cold chain independent and do not require refrigeration and so are suitable for proficiency testing at participating health centres and laboratories. They can also be used as an internal quality control.
Do I test my proficiency panel samples any differently than I test patient specimens?	No. Once reconstituted, the proficiency testing samples should be tested in the same manner as patient samples are tested.
May I discuss my proficiency testing results with another laboratory/ another healthcare worker?	You should not discuss your proficiency testing results with co-workers. Proficiency testing is an assessment of individual laboratories: clinics and testers should not know the sample status. Proficiency testing can help identify health care workers who may require additional support or training from the monitor/ supervisor. It is important that the results of the proficiency testing be honestly recorded by the health care worker who performed the test. They will be used to inform the lab director and internal monitor so that they can take the appropriate action.
Do I need to keep records of my proficiency testing?	Yes, you should keep a copy of the proficiency testing record form, in addition to any records detailing corrective actions performed at your laboratory or clinic. By keeping a record of corrective actions performed, similar problems can be prevented in the future.
What must I do if I do not get a passing score?	If a proficiency panel specimen fails, you will be informed by the central laboratory and steps should be taken to follow up the root cause of the problem. This does not mean that the health care worker does not know how to perform the test, but that the testing procedure was not optimal. Proficiency testing may have been done during hours when there was less light, there may not have been enough sample added, or there may have been an error in writing the test result in the correct column on the record form.
What is Incoming Inspection and why is it important?	Incoming inspection testing is carried out on each new lot or shipment of rapid test kits that arrive in-country from the supplier. It ensures the validity and integrity of test kits and lots after shipping. Testing can be carried out at the central or reference laboratory and at the health centre to re-confirm validity following shipment.
What should be tested during Incoming Inspection?	Test kits should be visually inspected to ensure that there has been no damage to the packaging or leakages. Lot/Batch numbers and expiry dates should be checked and there should be a visual check to ensure that all reagents and consumables are provided. In addition, to ensure that the test kits are still valid following shipment from the supplier, a representative number of test kits should be tested using a known negative or known positive control at the central or reference laboratory.

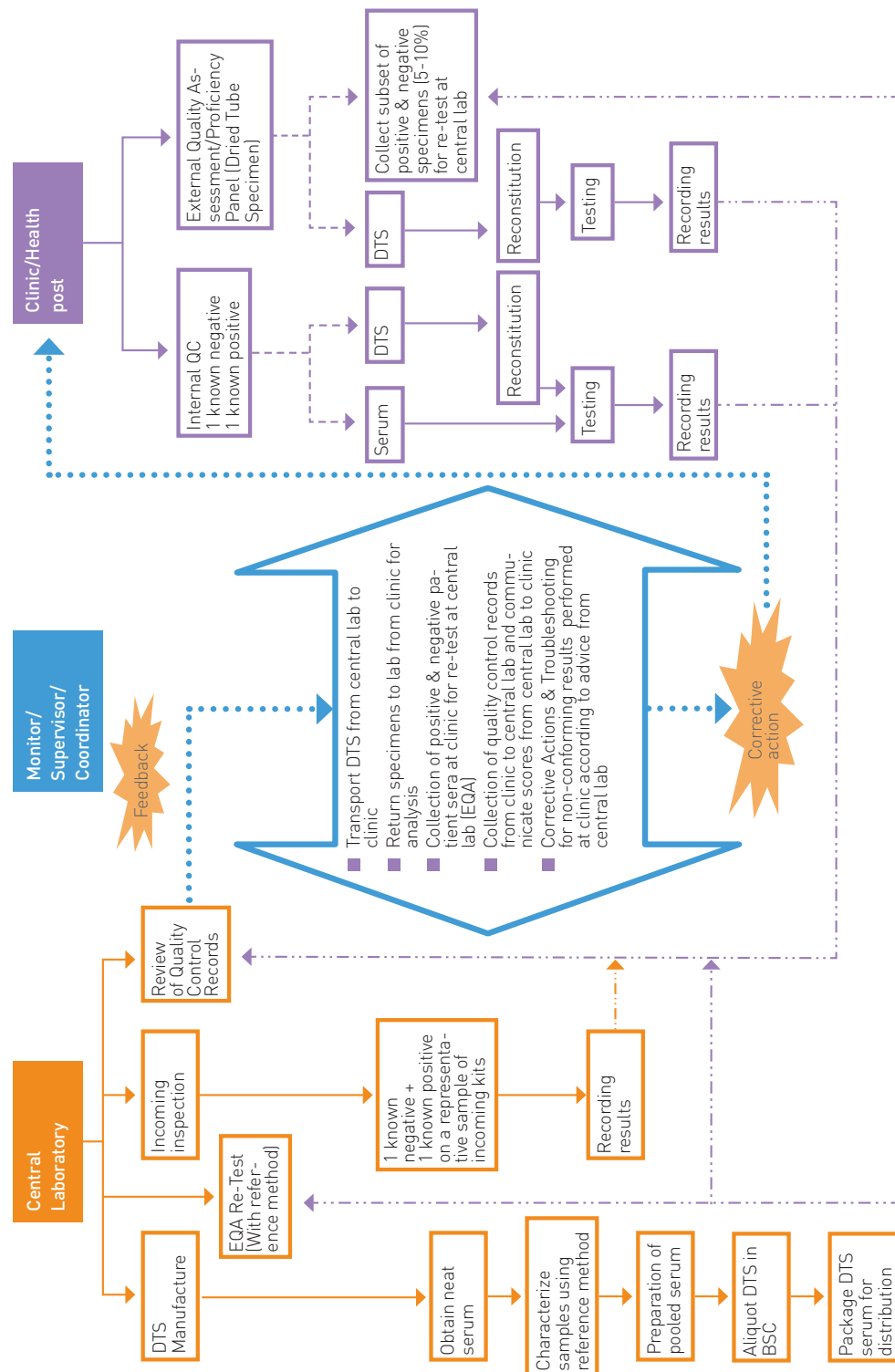
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- CDC/WHO, 2005. Guidelines for Assuring the Accuracy and Reliability of HIV Rapid Testing: Applying a Quality System Approach
- Manual on Quality Assurance for Laboratory diagnosis of malaria: Rapid Diagnostic Tests. Available from:
<http://nvbdcp.gov.in/Doc/SOP-Quality-Assurance-RDT.pdf>
- CLSI GP26-A2 “Application of a Quality System Model for Laboratory Services
- ISO 19011:2002 - Guidelines for Quality and / or Environmental Management System Auditing
- Clinical Laboratory Improvement Amendments (CLIA) regulation requirements brochure. Available at:
https://www.cms.gov/CLIA/05_CLIA_Brochures.asp#TopOfPage

Appendix 1. Overview of country approaches to a Quality Management System for a Rapid Syphilis Test programme

	Tanzania	Peru	Uganda	Brazil	Zambia
Internal Quality Control					
What type of sample is used for internal quality control at the clinic?	Dried Tube Specimen	Neat Patient Serum (liquid) + Dried Tube Specimen	Dried Tube Specimen	Neat Patient Serum (liquid)	Neat Patient Serum (liquid)
What is the frequency of internal quality control distribution to clinics?	Every month	Every month to every 2 months	Every week	Variable, depending on available transport	Samples were aliquoted into one use vials and distributed once to cover a 6 month period.
What is the mode of transport for distribution of internal quality controls from the central laboratory to the clinic?	Car	Via network coordinator by public transport	Car	Airplane and boat	Car
External Quality Control					
Materials for DTS Manufacture:					
What is the source of positive and negative samples?	Patient samples	Patient samples and volunteers	Blood donor clinic screened at public health laboratory	Samples from prison inmates	Commercial RPR control
What type of sample is obtained?	Serum	Whole blood and serum	Serum	Whole blood	Serum
Is RPR used to confirm serum sample status?	Yes	Yes	Yes	No	Yes
Is TPPA used to confirm sample status?	Yes	Yes	Yes	Yes	Yes
Is current test method (or test under evaluation) used to confirm sample status?	Yes	Yes	Yes	Yes	No
DTS Proficiency Pack Contents					
What is contained in the DTS Proficiency Pack that is sent to the clinic?	4 DTS vials, 1 x vial PT buffer 2 x plastic transfer pipettes 1 x instruction sheet, 1 x reporting form	2 DTS vials for IQC, 4 DTS vials for EQA 1 x vial PT buffer, 1 x plastic transfer pipette 1 x instruction sheet, 1 x reporting form Cassettes, gloves, capillaries, technopor rack	4 DTS vials 1 x vial PT buffer 5 x plastic transfer pipettes 1 x instruction sheet 1 x reporting form	6 DTS vials 1 x vial PT buffer 2 x plastic transfer pipettes 1 x instruction sheet 1 x reporting form	6 DTS vials 1 x vial PT buffer 2 x plastic transfer pipettes 1 x instruction sheet 1 x reporting form
How many schemes for distribution to health centres are planned per year?	6	2 - 12	4	Opportunistic	1
Other					
How many Dried Tube Specimens per proficiency panel?	4	2 - 4	4	6	6
How many vials of each panel member are prepared at one time at the central laboratory?	60	Maximum: 250	50	150	20
How long does it take to manufacture a batch of DTS specimens?	3 days	3 days	1 day	2 days	2 days
Who scores the record form?	Lab technicians at central lab	Internal monitor	Internal monitor/ District Lab supervisor	Lab technicians at central lab	Lab technicians at central lab
What is the cut-off average score below which corrective actions must be carried out?	≤75%	100%	≤ 75%	≤ 90	≤ 67%
What are the corrective actions when a health care worker scores less than the acceptable level of agreement stated in D6.13?	On-the-job re-training by internal monitor	Consultation/ Counselling Re-test using fresh reagents Re-training	Re-test using fresh reagents Re-training	Re-training	Re-training
What is the expiry date following DTS manufacture if specimens are kept at room temperature?	1 month	n/a	1 month	1 month	6 months
INCOMING INSPECTION					
Is incoming inspection performed at the central laboratory for each new shipment or lot of kits to ensure their validity?	Every lot	Every shipment	Every shipment	Not Performed	Every shipment
How many samples are tested for incoming inspection?	1 Positive, 1 Negative	1 Positive, 1 Negative, 1 weak positive	2 Positive, 2 Negative, 2 weak positive	n/a	4 Positive, 4 Negative
What is the representative number of test devices tested?	1% of all tests received	There is no record for this but it could be around 3-5%	1% of all tests received	n/a	1% of all tests received
Is a proportion of each kit lot/shipment retained at central laboratory?	No	Yes	Yes	n/a	Yes
If yes, what % of stock is retained?		1%	1 test kit	n/a	1%

Appendix 2. Summary flow chart of a Quality Management System in place for Rapid Syphilis Testing in country



Appendix 3. Example of a Standard Operating Procedure: Work instructions for use of standard diagnostics bioline syphilis 3.0 rapid test

Intended Use

The SD Bioline Syphilis 3.0 test is a solid phase immunochromatographic assay for the qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) against *Treponema pallidum*. This test method is intended for professional use as an aid in the diagnosis of syphilis.

Principle of the Procedure

The SD Bioline Syphilis 3.0 contains a membrane strip which is pre-coated with recombinant *Treponema pallidum* antigens (17, 15KDa) on the test band region. When the patient sample with sample diluent is added to the sample well, it moves with the recombinant *Treponema pallidum* antigen-colloid gold conjugate (17, 15KDa) along the membrane chromatographically to the test region (T) and forms a visible line as the antigen-patient antibody-antigen gold particle complex forms. The formation of a visible line in the test region (T) indicates a positive result for the detection of *Treponema pallidum* specific antibodies (IgG, IgA and IgM). When the *Treponema pallidum* specific antibodies (IgG, IgA and IgM) are absent in the sample, there is no visible colour band in the test region (T).

Kit Contents

- SD Bioline 3.0 test device
- Each test device contains colloidal gold conjugated to recombinant T. pallidum antigen (17, 15KDa) on test line and control line
- 1 bottle of Assay Diluent
- Disposable specimen droppers
- Instructions For Use

Materials required but not provided with the kit

- Gloves
- Timer or stopwatch
- Blood collection devices (lancets, capillary tubes, test tubes)

Storage and Stability

1. The test device should be stored at room temperature.
2. The test device is sensitive to humidity and heat. Perform the test immediately after removing the test device from the foil pouch.
3. Do not use beyond the expiration date
4. The shelf-life of the kit is indicated on the outer package.
5. Do not use the test kit if the pouch is damaged or seal is broken.

Precautions

1. The SD Bioline Syphilis 3.0 Test is intended for in vitro use. DO NOT RE-USE test device
2. The instructions for use must be followed exactly to give accurate results. Personnel performing the test must be trained in its use and must be experienced in laboratory procedures.
3. Collect whole blood using a suitable coagulant, and centrifuge whole blood to obtain plasma or serum specimen.
4. If specimens are not immediately tested, they should be refrigerated at 2 - 8°C. For storage periods greater than three days, freezing is recommended. They should be brought to room temperature before use.
5. Specimens containing precipitate may yield inconsistent test results. Such specimens should be filtered prior to assaying.
6. Whole blood may be used for testing immediately or may be stored at 2 - 8°C for up to three days.
7. Test results are not affected by anticoagulants such as ethylenediaminetetraacetic acid (EDTA), heparin or citrate.
8. Interference from haemolytic samples, rheumatoid factor-contained samples, lipemic samples and icteric samples can impair test results.
9. Use separate disposable pipettes or pipette tips for each samples in order to avoid cross contamination of samples, which could lead to erroneous results.
10. Do not eat or smoke while handling specimens.
11. Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
12. Avoid splashing or aerosol formation.
13. Clean up spills thoroughly using an appropriate disinfectant.
14. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials as if they were infectious waste in a biohazardous container.
15. Do not mix and interchange different specimens.
16. Care should be taken to avoid contamination of the end of the bottle when dropping assay diluent into sample well.

Quality Control

Good Laboratory Practice (GLP) requires the use of control specimens to ensure proper device performance at least once daily.

A built in procedural control on the test device indicates that the test is functioning correctly. A purple band should always appear at the control window.

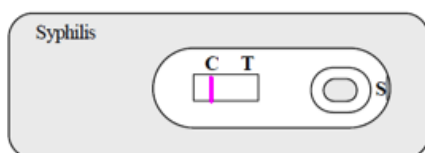
Internal and External controls should be run daily prior to analyzing patient/client specimens. Results should be recorded on the quality control log. Patient/client reports should only be reported if quality control results are acceptable.

Test Procedure

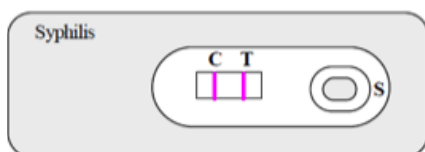
1. Remove the test device from the foil pouch, and place it on a flat, dry surface.
2. Transfer the specimen by pipette or dropper:
 - a. To use a pipette: Transfer 10µl of serum or plasma (or 20µl of whole blood) to the sample well (S) of the test device, then add 3-4 drops of assay diluent (approximately 110µl) and start the timer.
 - b. To use a Disposable Specimen Dropper: Hold the dropper vertically, draw the specimen (serum or plasma) up to the Fill Line (approximately 10µl). Transfer the specimen to the sample well (S) of the test device. In the case of whole blood, draw and transfer the specimen by the same method twice (approximately 20µl in total) and then add 3-4 drops of assay diluent (approximately 110µl) and start the timer.
3. As the sample moves chromatographically along the test membrane, a purple colour can be seen in the result window located in the centre of the test device.
4. The result should be interpreted within 5-20 minutes of addition of the sample. A positive sample will not change once it has been established after 20 minutes. However, in order to prevent any incorrect results, the result should not be interpreted after 20 minutes.
5. When whole blood is used, the test result should be interpreted within 10 minutes. Caution: The above interpretation time is based on reading the test result at room temperature. If room temperature is significantly lower than 10°C, the interpretation time should be extended to a further 10 minutes.

Interpretation of Test Results

6. A colour band will appear in the left section of the result window to show that the test is working properly. This band is the Control Band (C)
7. The right section of the result window indicates the test result. This is the Test Band (T).



Negative Result: The presence of only one purple colour band in the Control (C) region of the result window indicates a negative result.



Positive Result: If a colour band appears in Control (C) region and Test (T) region, the test result is positive for *Treponema Pallidum* antibodies.

Invalid result: If the purple colour band is not visible in the Control (C) region after the test has been performed, the result is deemed invalid. It is recommended that the specimen be re-tested.

Limitations of the Test

1. SD Bioline Syphilis 3.0 test procedure and interpretation of results must be followed closely when testing for the presence of syphilis antibodies in serum, plasma or whole blood.
2. The SD Bioline Syphilis 3.0 test will only indicate the presence of *Treponema Pallidum* antibodies in the specimen and should not be used as the sole criteria for the diagnosis of *Treponema Pallidum* infection.
3. As with all diagnostic tests, all results must be interpreted alongside other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of *Treponema Pallidum* infection.

Appendix 4. Protocol for preparation of dried tube specimen

1.0 Purpose

This procedure provides instructions on the manufacture of dried tube specimens to be used as a quality control as part of a proficiency testing programme.

2.0 Equipment and Materials

2.1 Equipment

- Multi-channel Pipettes
- Biosafety cabinet (BSC)
- Timer
- RPR Rotator

2.2 Materials

- 2.0mL conical bottom Sarstedt tubes
- Trypan Blue dye (0.1% stock solution)
- Pipette tips
- Disposable transfer pipettes
- Freezer boxes
- Tube racks
- Cryo labels
- Storage bottles
- Zip lock bags
- Labels
- 5mL disposable syringes
- Disposable filter unit 0.2µl
- Rapid plasma reagin kits
- *Treponema Pallidum* Particle Agglutination assay/ *Treponema Pallidum* Haemagglutination assay kits
- Phosphate buffered saline with Tween 20 (Sigma)

3.0 Handling Conditions

- Wear protective clothing while handling dried tube specimens
- Handle dried tube specimens as if capable of transmitting an infectious agent
- Do not interchange vial caps, as this may lead to cross contamination of specimens
- Leave the dried tube specimens in the biosafety cabinet (BSC) for overnight drying

4.0 Procedure

4.1 Plasma/Serum selection

- Obtain rejected plasma units from the local blood bank, sera from the diagnostic laboratory with a high syphilis titre (RPR titre $\pm 1:128$) and some RPR negatives. Store specimens at 2 - 8°C until further testing has been conducted.
- Verify the dilutions using *Treponema Pallidum* Particle Agglutination assay/ *Treponema Pallidum* Haemagglutination assay and rapid plasma reagin according to manufacturers' instructions and including positive and negative controls in the test run.

4.2 Dilution of serum/plasma

- Select the serum/plasma from a high titre source
- Titrate the serum using rapid plasma reagin, initially by 10-fold dilutions, then by 2-fold dilution in a negative serum
- Make a 4-fold dilution of the strongest positive serum in negative serum to yield a medium positive
- Make a 4-fold dilution of the medium positive in negative serum to yield a faint positive serum
- Note the dilutions giving these results
- Verify the dilutions using *Treponema Pallidum* Particle Agglutination test/ *Treponema Pallidum* Haemagglutination technique and rapid plasma reagin according to manufacturers' instructions and including positive and negative controls in the test run.
- Select dilutions that represent a high titred and a low tittered sample, based on the sample/cut-off ratios.

4.3 Preparation of Dried Tube Specimens

- 4.3.1** Prepare a 1:1000 dilution of Trypan Blue: Serum, e.g. add 1 μL of dye to 1 mL of specimen. Vortex the specimen to mix the dye.
- 4.3.2** Transfer 20 μL of Trypan blue - serum/plasma solution to each Sarstedt tube. Tubes should be labelled with specimen identification and expiry date.
- 4.3.3** Leave the tubes uncapped and allow to dry overnight in a biosafety cabinet, ensuring that different specimens are kept in separate racks in the BSC. The following day, ensure that all specimens are thoroughly dried before capping each tube.
- 4.3.4** A visible coloured pellet should have formed in the bottom of the tube. Store the capped dried tube specimens at 2-8°C until ready for shipping to participating laboratories.

4.4 Preparation of DST buffer (PBS/Tween-20)

4.4.1 Dissolve one foil pouch of phosphate buffered saline with Tween 20, Ph 7.4 in 1L of deionised water.

4.4.2 Filter the solution through a 0.2µm filter flask

4.4.3 Prepare 1.8mL aliquots of Proficiency Testing buffer in pre-labelled 2ml screw capped tubes

4.4.4 Label the tubes the identification “Proficiency Testing buffer”, with an expiry date of 1 year.

4.5 Preparation and Packaging of Dried Tube Specimen Panels

4.5.1 Create a panel of at least 6 samples from the characterized specimens with a combination of grades of reactivity for syphilis, including truly high titre positives and negatives.

4.5.2 Carefully blind the panel assigning a new identification (ID) to each sample, e.g. DTS-A1 to DTS-A6. Ensure there is traceability between the original ID and new ID.

4.5.3 Label each tube with the appropriate new ID.

Depending on the number of laboratories enrolled in the proficiency testing programme, prepare 10 to 20 extra sets and store at the central laboratory.

4.5.4 Proficiency panels for shipping to participating laboratories should contain:

- One member of each panel
- One vial of Proficiency Testing buffer
- Two plastic transfer pipettes (dropper)
- One instruction sheet
- One reporting form

4.5.5 Put all contents into a zip lock bag labelled with identification, expiry date and storage conditions.

4.5.6 The bagged Proficiency Panels can be stored at 2-8°C until shipment or delivery to testing sites.

4.6 Reconstitution of Dried Tube Specimens

4.6.1 Tap the dried tube specimen tube gently to ensure that the colored pellet falls to the bottom of the tube.

4.6.2 Using the dropper provided, add 7 drops of proficiency testing buffer to each dried tube specimen to be tested. Cover the tube, tap gently and leave overnight at room temperature.

4.6.3 The following day, mix the specimen by gently tapping the tube.

Test the re-constituted dried tube specimen with the appropriate syphilis tests.

Report the results using the report form provided.

4.7 Results Analysis

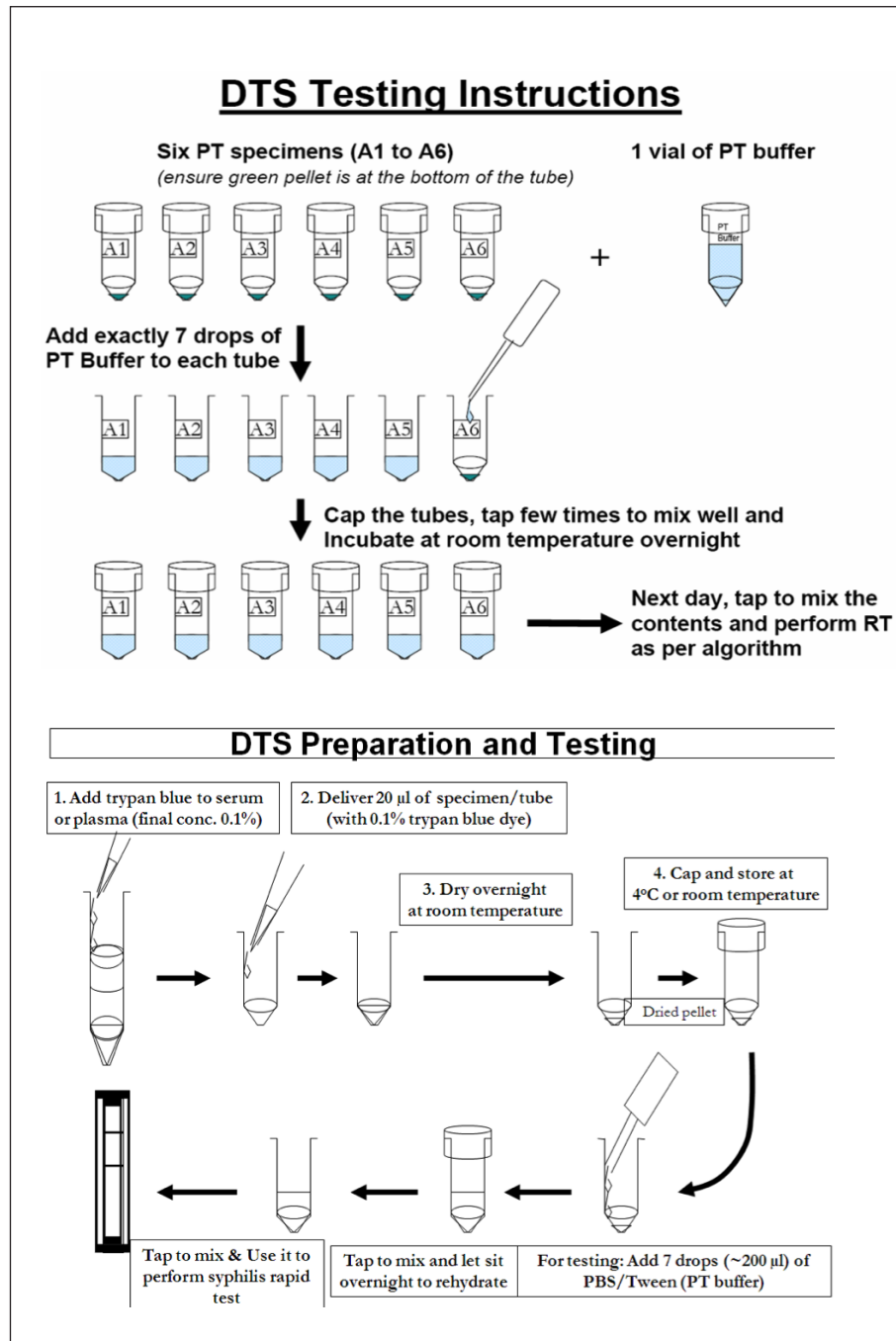
4.7.1 Collect report from all participating laboratories.

4.7.2 Enter data in the Excel spreadsheet.

4.7.3 Analyze the data and submit final report to all the participating laboratories.

4.7.4 Follow up with supervisor and/or additional training for those laboratories who do not receive a 100% agreement.

Appendix 5. Dried Tube Specimen Testing instructions Diagram



Appendix 6. Protocol for Preparing Syphilis – Positive Quality Control Materials

1.0 Purpose

This procedure provides instructions for making a supply of treponemal antibody positive samples at the desired reactivity level, to be used as a daily control or as part of a proficiency testing panel.

2.0 Equipment and Materials

2.1 Equipment

- Magnetic Stirrer, Non-Heated
- Single channel Pipettes (0.05 – 20 µl, 50-200 µl)
- Multi-channel Pipettes (0.5 – 20 µl and 50-300 µl)
- Vacuum Pump
- Tubing for Vacuum Pump
- Water bath or incubator
- Thermometer
- *Treponema Pallidum* Particle Agglutination assay/ *Treponema Pallidum* Haemagglutination assay/ Enzyme-linked immunosorbent assay (ELISA) equipment: e.g. reader, washer

2.2 Materials

- Unit (Blood Type Group O) of treponemal antibody positive serum
- Unit (Blood Type Group O) of treponemal antibody negative serum
- Sterilizing Filters, .22 micron
- Cryogenic vials, polypropylene, 1.0 ml (for storage of aliquots)
- Cryovial storage boxes
- Brain Heart Infusion (BHI) Broth (ready to use in tubes) or Brain Heart Infusion powder and materials to prepare tubes of broth.
- Sterile screw cap tubes, 16x125 (for Brain Heart Infusion broth, if needed)
- Non-sterile plastic tubes, polypropylene 12x75 (for serial dilutions)
- Glass Stir-rods
- Pipette tips
- Individually Wrapped Sterile Pipettes (1.0, 5.0, 10.0, 25ml)
- Discard or waste containers
- Disinfectant
- Autoclave bags
- Gloves and lab coats

3.0 Handling Conditions

- Units of treponemal antibody positive serum should be stored at 2 –8oC.
- Follow good laboratory safety practices when handling all samples
- Properly dispose of contaminated waste according to established waste disposal procedure.

4.0 Overview of Process

4.0.1 Calculate sufficient volume required for one year supply of ready-to-use aliquots, and an additional bulk volume for freezing and storing for future use, e.g., 250ml of serum will yield 500 aliquots of 0.5 ml.

4.0.2 Obtain treponemal antibody positive and treponemal antibody negative sera. Consider the National Blood Transfusion Service as one potential source of sera.

4.0.3 Heat inactivate positive sera and negative sera

4.0.4 Filter and sterilize positive and negative sera

4.0.5 Titrate treponemal antibody positive sera

4.0.6 To select desired titre of sample, perform *Treponema Pallidum* Haemagglutination technique/ *Treponema Pallidum* Particle Agglutination test/ Enzyme-linked immunosorbent assay test

4.0.7 Prepare bulk volume of selected titre

4.0.8 Validate results of bulk volume

4.0.9 Aliquot, label, and store

4.0.10 Perform homogeneity and stability testing

4.0.11 Maintain data logs and records

5.0 Stepwise Procedure

5.1 Calculate volume required

Calculate the total volume of sample required before beginning production to ensure that sufficient materials / reagents are available. The volume required may depend on a number of factors:

- How long the pooled serum is needed , e.g. 12 months
- How often the syphilis test is performed
- The sample volume required by the test
- The number of participating laboratories in your Proficiency Testing Programme
- Approximately 10% overage for determining homogeneity and stability testing

5.2 Obtain syphilis positive and negative sera

Obtain a unit of sterile treponemal antibody positive and treponemal antibody negative serum from Type O donors. Note: One unit yields approximately 400 ml of serum.

- Both units should be negative for Hepatitis B surface antigen (HBsAg) and HIV antigen.
- Both units should be non-haemolysed, non-lipaemic, and free of particulate material.
- The treponemal antibody positive unit should have a high *Treponema Pallidum* Haemagglutination technique/ *Treponema Pallidum* Particle Agglutination test/ Enzyme-linked immunosorbent assay antibody titre (6 – 8X test cut-off).

5.3 Heat inactivate treponemal antibody positive and negative sera

5.3.1 Heat-inactivate the treponemal antibody positive and negative units at 62°C for 20 minutes in a water bath.

5.3.2 If a water bath is not available, place the unit of serum inside a large glass container of water in an incubator set to 75°C for 20 minutes.

5.3.3 Place a thermometer in the water and monitor a rise in temperature to 62°C.

5.3.4 Place the unit of treponemal antibody positive serum in the water.

5.3.5 Continue to monitor the temperature of the water and when it again reaches 62°C, time for 20 minutes.

5.4 Filter and Sterilize sera

5.4.1 Using sterile technique, filter the heat-inactivated serum through a 0.22 micron sized sterile filter into a sterile enclosed polypropylene container.

5.4.2 Using sterile pipettes and sterile technique inoculate 3-4 tubes of Brain Heart Infusion Broth with 100µl of the heat-inactivated, filtered serum and incubate at 37°C for 7 days.

5.4.3 Store the remainder of the treponemal antibody positive heat-inactivated, filtered serum at 2-8°C.

5.4.4 Using sterile technique, filter the treponemal antibody negative serum through a 0.22 micron sized sterile filter into a sterile enclosed container.

5.4.5 Using sterile pipettes and sterile technique, inoculate 3-4 tubes of Brain Heart Infusion Broth with 100 µl of the filtered treponemal antibody negative serum and incubate at 37°C for 7 days.

5.4.6 Store the remainder of the treponemal antibody negative filtered serum at 2-8°C.

5.4.7 At the end of 7 days, check the broths for turbidity. If no turbidity exists in any tubes, begin titration of the treponemal antibody positive serum.

5.5 Titrate treponemal antibody positive sera (Determine desired dilution)

A titration is conducted as follows:

5.5.1 Make an initial 10-fold dilution of the antibody positive serum by adding 0.1 ml of the positive sera, and 0.9 ml of negative serum to tube 1.

5.5.2 Pipette 0.50 ml of antibody negative serum into tubes 2-12.

5.5.3 Make 2-fold dilutions in tubes 2-12 by mixing and transferring 0.50 ml of from tube 1 to tube 2.

5.5.4 Continue mixing and transferring 0.50 ml through the last tube, ending with a dilution of 1:20,480.

5.6 Perform a *Treponema Pallidum* Haemagglutination assay/ *Treponema Pallidum* Particle Agglutination assay or Enzyme-linked immunosorbent assay test to select desired titred sample

5.6.1 Perform a *Treponema Pallidum* Haemagglutination assay/ *Treponema Pallidum* Particle Agglutination assay/ treponemal Enzyme-linked immunosorbent assay following the manufacturer's instructions and your standard operating procedure.

5.6.2 In accordance with the manufacturer's instructions, include kit positive and negative controls in the test run.

5.6.3 Test each titrated sample, and the original sample from which dilutions were made, in triplicate.

5.6.4 Based on the Sample/Cut-off ratios obtained in Enzyme-linked immunosorbent assay tests or the titre obtained in *Treponema Pallidum* Haemagglutination assay/ *Treponema Pallidum* Particle Agglutination assays calculated for the treponemal antibody positive diluted samples, select dilutions that represent a high titre and a low titre sample. Generally, a low titre positive has an S/Co ratio of 2 – 3 and a high titre positive has an S/Co ratio of 5 – 6 when ELISA testing is used and titres of 1:160 – 1:320, and 1:1280 – 1:2560.

5.6.5 Plot the results obtained against the sample dilutions and calculate appropriate dilutions to obtain low and high titre positive sera.

5.7 Prepare Bulk Volume

5.7.1 Choose the appropriate dilutions for high titre and low titre positive samples to determine the volume of sample suitable for your purpose. For example, if 500 ml is needed and the 1:2560 dilution was selected as the high titered sample, add 200 µl of the bulk antibody positive serum to 512 ml of the antibody negative serum. Use a sterile container with a lid to contain the dilutions for the bulk samples.

5.7.2 Add a preservative such as Bronidox (0.5%), to the final diluted serum, e.g. 0.5mls to 500mls of diluted control. Check the package insert of the assay for which the quality control/ proficiency testing sample is to be used, to ensure that the preservative is appropriate and will not interfere with the performance of the assay.

5.7.3 Place the diluted serum on a magnetic stirrer in a biohazard cabinet and mix for at least one hour to ensure homogeneity of the diluted serum.

5.8 Validate results of pooled serum

Re-test using the same treponemal assay to validate the results of the diluted batches of serum. Compare these results of the batch with the initial *Treponema Pallidum* Haemagglutination technique/ *Treponema Pallidum* Particle Agglutination test/ Enzyme-linked immunosorbent assay results.

5.9 Aliquot, Label, and Store

5.9.1 If results of the re-test are acceptable, aliquot appropriate (small working) volumes, e.g. 0.5-1.0 ml from the high positive and the low positive batches into sterile internal threaded, polypropylene vials with silicon O-rings in the caps.

5.9.2 Store the vials and remaining bulk volume in well labeled containers at -80°C until needed. Aliquots stored at 4°C should be discarded after one week.

5.10 Perform homogeneity and stability testing

5.10.1 To ensure that the pooled serum has been well mixed and homogenous, randomly select approximately 10% of total aliquots. Test these samples, and compare results with target titres. The batch of pooled serum is acceptable if the Coefficient of Variation of the results is less than 15%.

5.10.2 To validate the stability of the level of reactivity of the pooled serum, place aliquots of the sample at -20°C, 4°C, and room temp (15-25 °C), Test the samples at 7, 14, 21, and 28 days. Review the results obtained at each temperature. The batch of pooled serum is considered stable if the titres fall within ± 2 standard deviations of the original results.

Appendix 7. Daily Record of Quality Control Results

Kit: _____ Lot #: _____ Expiration Date: _____

[illegible]

Corrective Actions

[illegible]

Appendix 8. – External Quality Assessment of Syphilis Rapid Tests: Operational issues for re-testing

If re-testing is used as a way to conduct external quality assessment for rapid syphilis testing, there are a number of aspects to be considered when determining and implementing national (or local) policy.

Collection of samples for retesting

Appendix 8 provides tables and guidance for the selection of the appropriate sample size for re-testing. The numbers shown in the tables can be applied to any time period: countries will need to select the appropriate period, balancing the need for a reasonable re-test sample size against how frequently performance needs to be evaluated. For example, opting to do yearly re-tests may make it feasible for a reference laboratory to test the required number of specimens, but it seems unreasonably spread out for the purpose of detecting potential performance problems. An interval of three months between re-tests may, in smaller sites, result in a very large number of repeat specimens, but it would provide a more frequent snapshot of the performance of the site. In summary, the re-test sample size required for statistical validity and the time period to be used for measurement must be determined based upon practicality and sustainability.

Wherever possible, the samples collected for re-testing should be randomly selected and distributed throughout the testing period. This will allow the re-testing external quality assessment to be more representative of the testing process.

In a site where multiple staff perform testing, it is logistically difficult to arrange to have a separate sample for each individual. But if the re-testing is to be representative of the performance of the entire site (i.e. of all persons who perform testing), it is important to ensure that samples collected for re-testing represent as many staff members as possible.

In some areas, a venous specimen is collected for testing with a rapid plasma reagin or rapid syphilis test kit. In this instance, there can be retrospective selection of specimens for a random sample.

When this method is used, all specimens may be aliquoted and stored. Later, a random selection process is used to determine which specimens to re-test. Alternatively, the selection may be done first, and only the selected specimens aliquoted and stored.

Venous blood collection may also be carried out at sites that perform syphilis rapid testing with a finger prick. In this case, prospective identification of the clients to be tested is required.

Recording information and transporting re-testing samples

It is important to maintain patient confidentiality in the re-testing process. It is recommended that a laboratory register or specimen number be used when sending samples from the original testing site to the reference laboratory for retesting.

Distributing the re-testing workload for the reference laboratory will help to avoid work overload and delays in returning results to the testing site. This will require a preplanned schedule to ensure that re-test specimens reach the reference laboratory at spaced intervals. Frequent transport, a continuous re-testing process and prompt feedback of results will help to assure timely monitoring of performance and prompt alerts when problems are detected.

Finally, care must be taken to assure that specimens are transported in such a way that the reference or re-testing laboratory receives them in good condition.

Re-testing of samples

Generally, re-testing of samples will be carried out by one or several reference laboratories within the country. When dealing with venous blood, the reference laboratory or laboratories should assure quality of testing by appropriate validation of the Enzyme Immuno Assay or *Treponema Pallidum* Haemagglutination/ *Treponema Pallidum* Particle Agglutination technology employed. In addition, all laboratories performing this reference testing should participate in external quality assessment for syphilis testing.

Following re-testing, it is necessary to define your approach to the investigation of any discrepancies between the original rapid testing and the comparator treponemal laboratory test. Errors or differences in results may occur for a variety of reasons. Operator error in test performance is one cause of discrepancy, and this will require additional quality assurance and training at the site. Another common source of error is a transcription mistake at some point in the process. Errors may be produced if the samples for re-testing are improperly stored and/or transported. Very slight differences are also observed between rapid tests and comparator tests, with neither being more accurate than the other. All discrepancies require investigation. A policy must be developed for the resolution of discrepancies, and an acceptable level of discrepancy must be determined.

Reporting and corrective action

The results of the re-testing should be reported to the original testing site and to the designated quality officer where applicable. The quality officer at the testing site should evaluate all results received from re-testing, and take appropriate corrective action when performance goals are not met.

In most countries, the Ministry of Health will also be a recipient of information on the results of re-testing. Results should be collected systematically and used to evaluate testing performance on a national basis, as well as to initiate appropriate corrective action when needed.

Appendix 9. External Quality Assessment of Rapid Syphilis Tests Statistical Models for Re-testing

External Quality Assessment of Syphilis Rapid Tests: Statistical Models for Re-testing

Re-testing of samples has been used to monitor rapid syphilis testing in lieu of conventional External Quality Assurance or proficiency testing, which is often unavailable to laboratories and testing sites. This document looks at the statistics that apply to this re-testing, and provides information that will be useful in determining appropriate models for external quality assessment re-testing.

Current Situation

Currently there are a variety of external quality assessment re-testing schemes in place in various countries. Examples include:

- Re-testing 5% of all samples and the first 40 samples tested by each technician who runs tests.
- Re-testing 10% of all samples.
- Re-testing all positives and varying percentage of the negatives.

Other considerations are as follows:

- In all cases re-testing is done with a confirmatory treponemal laboratory test (*Treponema Pallidum* Haemagglutination technique, *Treponema Pallidum* Particle Agglutination test or Enzyme-linked immunosorbent assay).
- Current re-testing schemes do not account for the number of samples tested at each site. This can vary widely, between 50 and 1000 tests per month, or between 500 and 10,000 per year.
- Current national rates of syphilis prevalence vary from low to very high. The rate of positivity may be much higher (or lower) than the national prevalence in a particular site. Rate of positivity in testing sites is highly variable.
- Data on the agreement between rapid syphilis testing methods is becoming more widely available as national rapid syphilis test evaluations are taking place. The algorithms used require confirmation of all positive tests by at least one different method. In many cases, negatives also have to be negative on two kits (in some countries initial negatives are not confirmed). In cases where the first two results do not agree, a third kit is used and this is considered confirmatory (several variations exist, but this is sufficient for the purposes of this discussion).
- The sample for re-testing must be obtained at the time of initial testing.

Other considerations (continued):

- Errors (disagreement with comparator laboratory tests) can occur for a variety of reasons, including
 - Use of outdated kits
 - Improper storage of kits
 - Lack of technical competence
 - Clerical error
 - Insensitivity of the kit
- Information on current agreement rates for re-tested samples is not available for use in this document. It would be very useful to know the distribution by agreement on positive and negative samples, the agreement rates at different sites, and the agreement rate for new technicians, in addition to the overall agreement rates that are found

Objectives

The model should consider the following variables:

- True error rate (unknown)
- Positivity rate
- Population size (number of cases per study period)
- Probability of detection of errors
- Number of re-tested cases, or the proportion of cases to be re-tested
- Decision rule: act on a single discrepancy or multiple discrepancies?

The recommended re-testing scheme should achieve the following objectives:

- Provide a stated level of confidence that low error rates will be detected.
- Be independent of positivity rate.
- Accommodate different numbers of tests performed in the time period (50-10,000).
- Assume that even a single disagreement will lead to investigation.
- Assume that re-testing is performed without error.

Model Assumptions

The model presented below could be applied equally to samples that are positive and negative on initial testing; or it could be used on all tests, no matter what the initial result. The recommended quantity of samples could be drawn independently from patients who are initially diagnosed as positive and from those that are initially diagnosed as negative; or it could be chosen randomly from all patients. In some instances it would be difficult to base re-testing on the initial result, so the easiest re-testing scheme would come from a random sample of all patients.

The model assumes that any discrepant result is a signal for action. Whatever the actions are for “suspect” results from test sites, these actions would be initiated on the discovery of a single discrepant result, no matter what the sample size or positivity rate.

The suggested model eliminates the need to consider the rate of syphilis seropositivity, but there remain four important dimensions to the recommendations:

- Population size
- Sample size
- True error rate
- Confidence level

Positivity rate would not affect the estimates in the following tables and figures, but it would affect the “power” of the procedure (its ability to detect errors) if there are different probabilities for false negatives and false positives. The positivity rate and the clinical impact of false positives and false negatives could lead to different re-testing procedures for positives and negatives.

Model

The hypergeometric distribution can be used to predict the probabilities of detection for any given sample size. In this model:

- Eight different sizes of tests were checked (this could be numbers of negatives or positives, or both): 50, 100, 200, 500, 1,000, 3,000, 5,000, and 10,000.
- Three different possible error rates were investigated: 1%, 3%, and 5%.
- Three levels of confidence were checked: 90%, 95%, and 99%.
- Three different re-testing rates were checked: 5%, 10%, and 20%.
- For small samples and low error rates it was necessary to assume at least 1 error; for example, 50 samples with 1% error rate was assumed to have 1 error, and a 5% sample produces 3 cases. This can distort the percentages in the tables.

The model was applied in three different ways to answer three questions:

1. For given numbers of cases and given error rates, what sample size is needed to ensure a stated confidence of having at least 1 discrepant result? These numbers can then be converted to percentages of the number of cases. (See Table 1a-c.)
2. For given numbers of cases, given re-testing rates and given error rates, what is the probability of observing at least one discrepant result? This can also be called the Power of the re-testing and decision rule. (See Table 2a-c.)
3. For given numbers of cases and given re-testing rates, what is the lowest error rate that can be detected with a stated confidence? This is the upper limit of the Confidence Interval for the error rate (the lower limit is zero). (See Table 3a-c.)

TABLE 1a: Re-test size (and %) needed to provide 90% confidence of detecting at least one discrepant result, when the underlying error rate is 1%, 3%, or 5%E

Number	1%E	3%E	5%E
50	45 (90%)	34 (68%)	27 (54%)
100	90 (90%)	54 (54%)	37 (37%)
200	137 (64%)	63 (32%)	41 (21%)
500	184 (37%)	71 (14%)	43 (8.6%)
1000	205 (21%)	73 (7.3%)	44 (4.4%)
3000	221 (7.4%)	75 (2.5%)	45 (1.5%)
5000	224 (4.5%)	76 (1.5%)	46 (.92%)
10000	227 (2.3%)	77 (0.77%)	47 (0.47%)

Example: If there are approximately 1,000 cases in the time period, and 90% confidence is acceptable for detecting 5% errors, then a 4.4% re-test will suffice (44 samples).

TABLE 1b: Re-test size (and %) needed to provide 95% confidence of detecting at least one discrepant result, when the underlying error rate is 1%, 3%, or 5%E

Number	1%E	3%E	5%E
50	48 (96%)	39 (78%)	31 (62%)
100	95 (95%)	63 (63%)	45 (45%)
200	155 (78%)	78 (39%)	51 (26%)
500	225 (45%)	90 (18%)	56 (11%)
1000	258 (26%)	94 (9.4%)	57 (5.7%)
3000	284 (9.5%)	97 (3.2%)	58 (1.9%)
5000	290 (5.8%)	98 (2.0%)	59 (1.2%)
10000	294 (2.9%)	99 (1.0%)	60 (0.60%)

Example: If there are 200 cases and the objective is to have 95% confidence in detecting an error rate of 3% or more, then the number of re-tested cases would be 78, or 39% of all cases.

TABLE 1c: Re-test size (and %) needed to provide 99% confidence of detecting at least one discrepant result, when the underlying error rate is 1%, 3%, or 5% E

Number	1%E	3%E	5%E
50	50 (100%)	45 (90%)	39 (78%)
100	99 (99%)	78 (78%)	59 (59%)
200	180 (90%)	106 (53%)	73 (37%)
500	300 (60%)	131 (26%)	83 (17%)
1000	368 (37%)	141 (14%)	86 (8.6%)
3000	425 (14%)	148 (4.9%)	88 (2.9%)
5000	438 (8.8%)	149 (3.0%)	89 (1.8%)
10000	448 (4.5%)	150 (1.5%)	90 (0.90%)

Example: If it is desired to have 99% confidence that an error rate of 1% or more can be detected, in a situation with 50 (or fewer) cases, then 100% of results need to be re-tested.

TABLE 2a: Probability of obtaining at least one discrepant result with re-testing rates of 5% with error rates of 1%, 3%, and 5%E

Number	1%E	3%E	5%E
50	.06	.12	.17
100	.05	.14	.23
200	.10	.27	.41
500	.23	.54	.73
1000	.40	.79	.93
3000	.79	.991	1.0
5000	.92	1.0	1.0
10000	.994	1.0	1.0

Example: If there are 5,000 cases and a 1% error rate (50 errors), then if 5% of cases are re-tested (250 cases) there is a .92 probability of selecting at least one of the errors (power). If there are 1000 cases (50 re-test cases), there is a .93 chance of detecting 5% errors.

TABLE 2b: Probability of obtaining at least one discrepant result with re-testing rates of 10% with error rates of 1%, 3%, and 5%E

Number	1%E	3%E	5%E
50	.10	.19	.28
100	.10	.27	.42
200	.19	.47	.66
500	.41	.80	.93
1000	.65	.96	.996
3000	.96	1.0	1.0
5000	.995	1.0	1.0
10000	1.0	1.0	1.0

Example: If there are 1,000 cases, an error rate of 3% and a re-test rate of 10%, then there is a probability of .96 that at least one result will be discrepant.

TABLE 2c: Probability of obtaining at least one discrepant result with re-testing rates of 20% with error rates of 1%, 3%, and 5%E

Number	1%E	3%E	5%E
50	.20	.36	.50
100	.20	.49	.68
200	.36	.74	.90
500	.67	.97	.997
1000	.89	.999	1.0
3000	.999	1.0	1.0
5000	1.0	1.0	1.0
10000	1.0	1.0	1.0

Example: If there are 500 cases and a 20% re-sampling (100 re-sample cases), then there is a .67 probability of having at least one failure in the sample when the error rate is 1%.

TABLE 3a: Lowest error rate that can be detected with 90% confidence with the stated re-testing rates (%ReT) and given number of cases

Number	5%ReT	10%ReT	20%ReT
50	54%	36%	20%
100	37%	20%	10%
200	21%	11%	5.5%
500	8.6%	4.4%	2.2%
1000	4.4%	2.2%	1.1%
3000	1.5%	0.73%	<.5%
5000	0.90%	<.5%	<.5%
10000	<.5%	<.5%	<.5%

Example: With 3,000 cases, it would require 10% re-testing (300 re-test cases) to detect a 1% error rate (table entry 0.73%), with 90% confidence. If no errors are found, the 90% confidence interval for the error rate is (0 to 0.73)

TABLE 3b: Lowest error rate that can be detected with 95% confidence with the stated re-testing rates (%ReT) and given number of cases

Number	5%ReT	10%ReT	20%ReT
50	62%	44%	24%
100	45%	25%	13%
200	26%	14%	6.5%
500	11%	5.6%	2.8%
1000	5.7%	2.9%	1.4%
3000	1.9%	0.97%	<.5%
5000	1.2%	0.58%	<.5%
10000	0.58%	<.5%	<.5%

Example: If 10% of 50 slides are re-tested (5 re-test cases), and no errors are found in the sample, then the 95% confidence interval for the error rate is: (0 to .44).

TABLE 3c: Lowest error rate that can be detected with 99% confidence with the stated re-testing rates (%ReT) and given number of cases

Number	5%ReT	10%ReT	20%ReT
50	90%	78%	34%
100	59%	36%	19%
200	37%	20%	10%
500	17%	8.4%	4.2%
1000	8.6%	4.3%	2.1%
3000	3.0%	1.4%	0.70%
5000	1.8%	0.88%	<.5%
10000	0.90%	<.5%	<.5%

Example: With a 20% re-test rate and 200 slides in the population (40 cases selected), the error rate has to be at least 10% (20 errors), if we are to have 99% chance of including at least one of the errors in the re-tested cases. If no errors are found in the re-test cases, the 99% confidence interval for error is (0 to .10).

Observations

1. The estimates above can be applied to any subset of testing situations, or any combined group, including all tests done in a year or all tests done with a specific kit. The numbers in Tables 1-3 can be used to estimate the sample sizes needed to assure levels of performance of specific testing centres, technicians, or kits.
2. Traditional proficiency testing and re-testing are both useful external quality assessment methods; they serve similar purposes in some ways, but differ in their ability to detect errors and in the services they provide. Both systems:
 - Monitor performance to detect systematic errors.
 - Motivate laboratory and technician to pay attention to quality.
 - Assure responsible oversight.
 They differ in that:
 - Re-testing provides more samples than proficiency testing and is therefore more sensitive to errors. Since error rates of concern are expected to be <5%, large samples are required to detect errors.
 - Proficiency testing provides controlled samples and routine interlaboratory communications, with manageable operation.
3. Current field data or re-test data should be mined for additional information, such as agreement between kits and the numbers of “tie-breakers” required by a technician or a facility. These could be important quality indicators. For example, there should be routine recording of all tie-breaker cases, including kit names (and lots) and tie-breaker result.
4.
 - a. In sites with low numbers of cases (<500), the likelihood of detecting errors is very low unless large percentages are re-tested. This would apply to programmes that require re-testing of all positives (low error rate, high power required).
 - b. In sites with 500 or more cases there are opportunities for reasonable power for error detection, with feasible but large numbers of re-test cases.
 - c. In situations involving very large numbers of cases (3,000 or more), the number of re-tested cases can be capped. A re-test of 200 to 250 cases would seem to provide high power for detecting low error rates, so re-testing rates could be set accordingly.

Appendix 10. Example specimen Transfer log for re-testing

[Insert Name of Referring Testing Site, Contact Name, Address and Phone Number]

Date:		Referring Test Site:				Contact Name:			
Address:						Telephone:			
Specimen Tracking Number	Test Sub-ject ID*	Final Result (Testing Site)	Date Specimen Collected	Specimen Type (DBS or Serum)	Collected by	Referral Lab Req [†] Completed (✓)	Date to referral lab	Date Conf Result Received	Result of Re-test

*ID = Identification
†Lab Req = Laboratory Requisition

Appendix 11. Daily Temperature check chart

Daily Temperature Check Chart for Refrigerator/Freezer/Incubator #:			
Thermometer #:		Temp Set:	Month: Year:
Date	Temp Observed	Initials	Comments
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			
Supervisor (initials):		Name:	Date:
Binder #		Storage Location:	

Appendix 12. Protocol worksheet used in Brazil

TESTES RÁPIDOS EM ÁREA INDÍGENA

PROTOCOLO DE BANCADA

SÍFILIS SD 3.0
Amostra: Sangue Total
Quantidade: 01 marca da pipeta ($\pm 20 \mu\text{l}$)
Tampão: 04 gts.
Tempo: 15 min.

BIOMANGUINHOS
Amostra: Sangue Total
Quantidade: 01 alça ($\pm 5 \mu\text{l}$)
Tampão: 03 gts.
Tempo: 10 min.









**FLUXOGRAMA PARA
DIAGNÓSTICO DA
SÍFILIS
PELO TESTE RÁPIDO**



**FLUXOGRAMA
PARA TRIAGEM DO
HIV
PELO TESTE RÁPIDO**



Appendix 13. Pictorial diagram of the manufacture of DTS in Brazil

FUNDAÇÃO ALFREDO DA MATTA
 PROJETO: "Acesso progressivo à qualidade: Diagnósticos seguros de sífilis em localidades remotas da Amazônia"
CONTROLE DE QUALIDADE EXTERNO DOS TESTES RÁPIDOS PARA DIAGNÓSTICO DA SÍFILIS E DO HIV – Utilizando DTS (Dried Tube Specimens)

ATENÇÃO: Realizar os passos 1, 2 e 3 no dia anterior à execução do teste.

Passo 1

Colocar os tubos em superfície lisa e plana, verificando a existência de um precipitado de cor azul no fundo de cada tubo.

Passo 2

Abriu o tubo contendo a amostra de DTS (A1, A2, ..., A6) e adicionar 7 gotas ($\approx 200 \mu\text{l}$) de tampão PT, em cada tubo, com auxílio da pipeta de transferência de plástico tipo Pasteur.

Passo 3

Tampar o tubo, bater suavemente com o dedo no fundo. O sedimento de cor azul se desprenderá, dando lugar a uma solução azulada (clara). Guardar os tubos contendo as amostras DTS (A1, A2, ..., A6) no saco plástico de origem, em temperatura ambiente, por uma noite.

Passo 4

No dia seguinte...
...misturar a amostra batendo suavemente, com o dedo, no fundo do tubo.

Passo 5

Realizar os testes rápidos de sífilis e HIV utilizando as amostras de DTS reconstituídas.

Passo 6

Interpretar os resultados obtidos, anotando-os na planilha.

Appendix 14. Pictorial instructions on performing rapid syphilis testing (in Brazil)

Instruções para execução do TESTE RÁPIDO DE SÍFILIS

1

Limpar com álcool o dedo do paciente que será utilizado para a coleta do sangue.

2

Após o álcool secar, furar o dedo do paciente para coleta do sangue.

3

Coletar o sangue até a marca indicada na pipeta que acompanha o pacote.

4

Depositar uma (01) gota do sangue, encostando a pipeta na área da amostra (S).

5

Adicionar na área da amostra (S), quatro (04) gotas do tampão.

6

Aguardar 15 minutos para a leitura.
NÃO ESQUEÇA DE ANOTAR O RESULTADO NO PROTOCOLO DE BANCADA.

ATENÇÃO
 O TESTE SERÁ CONSIDERADO INVÁLIDO SE, APÓS 15 MINUTOS, NÃO SURTIR A BARRA VERMELHA NO CONTROLE (C). NESSE CASO, O TESTE DEVERÁ SER REPETIDO

POSITIVO

Se o resultado for POSITIVO, aparecerão duas barras vermelhas no recipiente retangular: uma ao lado da letra C e outra ao lado da letra T.

NEGATIVO

Se o resultado for NEGATIVO, aparecerá apenas uma barra vermelha no recipiente retangular ao lado da letra C.