World Health Organization

Validation of EMTCT of HIV and/or syphilis
Tools and checklists for in-country evaluation of four required components

3		**Laboratory evaluation and assessment** **Guidance, checklists and tools**

I. Introduction

In 2014, the World Health Organization (WHO) released the global and regional criteria and processes for validation of elimination of mother-to-child transmission (EMTCT) of HIV and syphilis ("Global guidance on criteria and processes for validation: elimination of mother-to-child transmission of HIV and syphilis"). In addition to outlining the impact and process indicators for validation, the document highlighted the importance of ensuring that other key aspects of the EMTCT programme are assessed, such as the quality of data and laboratory systems. The present document is intended to provide operational guidance on how to assess a laboratory system for the purposes of validation of EMTCT of HIV and syphilis. This document should be sent to the country requesting validation of EMTCT prior to the visit of the validation mission team[1] to allow the national and subnational laboratories time to prepare data in advance for the laboratory assessment team to verify during the inspection.

Assessment of the programme and facilities is necessary because many of the laboratory-based HIV and syphilis tests require considerable infrastructure, as does maintaining quality assurance (QA). As expected, this can be challenging in resource-limited countries.

In addition, there are a number of technical complexities around the testing of HIV and syphilis in pregnant women and their infants that need to be considered in the assessment of national programmes. A positive HIV diagnosis in the mother requires the use of at least two, and often three, different serological tests. By contrast, the infant testing algorithm typically consists of an initial series of virological tests, and if these are negative for HIV, a final confirmatory serological test at 18 months. For syphilis, there are both treponemal (Tp) and non-treponemal (NTp) tests to make a diagnosis. The former is specific for syphilis but does not discriminate between current and past infection, whereas for the latter, antibody titres vary with the stage of infection, but may be falsely positive especially when titres are low. Furthermore, the algorithms used to determine HIV and syphilis status in mothers and infants vary widely from

[1] This could be either a prevalidation or validation mission.

country to country and use different test kits with different laboratory procedures. As a result, a detailed and thorough assessment of laboratory testing systems and processes is essential to determine whether national programmes are accurately measuring maternal and infant syphilis or HIV.

Finally, this tool is not meant to replace the WHO Stepwise Laboratory Quality Improvement Process Towards Accreditation (SLIPTA) but to complement it. It is highly recommended that laboratories apply for SLIPTA as a quantitative measurement of the quality of their laboratories, and to provide data and a basis for continuous laboratory improvement. SLIPTA is a comprehensive process that extends beyond HIV and syphilis testing, and participation in SLIPTA will facilitate and help to prepare a country for EMTCT laboratory validation.

This document outlines the methodology for the laboratory assessment, such that all countries seeking validation are assessed using a common set of standards. The proposed methodology draws from existing WHO guidance for laboratory audits, laboratory quality improvement and accreditation of the Pan American Health Organization, and the International Organization for Standardization (ISO) 15189 standards.

The main objectives of laboratory assessment are:

- to verify the existence of an adequate laboratory network to provide the services needed to achieve and maintain a programme for EMTCT of HIV and syphilis; and
- to ensure that the results generated by the laboratory network are accurate and reliable.

The proposed assessment consists of four principal steps:

(1) assessment of the overall performance of the national laboratory system in supporting the diagnosis and appropriate management of HIV and syphilis;
(2) observation of specimen collection, equipment, and HIV and syphilis testing procedures in testing facilities;
(3) team communication on the day's observations; and
(4) synthesis of data (observations and interviews) and dissemination of findings.

Mechanism for global and regional validation of elimination targets

The national validation committee (NVC) is responsible for coordinating an initial preparatory assessment that involves the collection and analysis of the national data and its assembly into a country report. This information is in turn evaluated by a regional validation committee (RVC), which is responsible for reviewing the country data, conducting validation visits to the country and assembling the regional validation report for submission to the global validation advisory committee (GVAC). A GVAC consisting of a multidisciplinary team of independent experts is mandated to ensure that the regional validation reports are compliant with the global elimination strategy and criteria, develop and evaluate validation tools, develop and

summarize recommendations concerning validation, and provide advice and guidance to countries on elimination activities and through the validation process.

PART II: IMPORTANCE OF LABORATORY DATA ASSESSMENT

1. Rationale for validation of laboratory data

As HIV infection and syphilis are asymptomatic and require screening using a diagnostic test, it is critical that countries have a robust laboratory network and that pregnant women have access to screening at all levels of the health-care system. The quality of the tests and of testing must meet appropriate quality control (QC) and QA mechanisms. The quality of the laboratory data is determined by the quality of the laboratory services that governs them. A deficient laboratory service will lead to unreliable surveillance data and incorrect diagnosis, which in turn will prevent appropriate treatment, compromising control and elimination efforts.

In countries with limited laboratory infrastructure, access to screening can be provided by the use of rapid point-of-care (POC) tests) at antenatal clinics. The use of POC tests for screening of syphilis and HIV infection has been expanding. These tests are used in non-laboratory settings by staff with no formal laboratory training. Appropriate quality management systems need to be in place to ensure the quality of the POC tests and the proficiency of staff that performs them. QA for POC tests is feasible, but can present challenges, as testing is decentralized from a few laboratories to hundreds of POC test sites in a country.

2. Quality of tests

Test selection

Countries should be able to show that the test(s) selected for use in a disease control and/or elimination programme has acceptable performance and operational characteristics as specified by national and international organizations such as WHO, the United Nations Children's Fund (UNICEF), Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund), and the United States Agency for International Development (USAID) Waiver List. In the absence of global consensus, countries should show evidence that the tests selected have been evaluated against an external reference standard and shown to detect the target of interest with high sensitivity and specificity.

The selection of POC tests is an important feature for most disease control programmes in order to increase access to testing. How these tests are managed in combination with laboratory tests should be documented.

Test procurement and supply chain management

Testing coverage can be adversely affected by stock-outs. Evidence of good procurement practice and sound national supply chain management is essential to ensure the quality of tests across the country and to avoid stock-outs. Lot testing performed nationally post purchase and before distribution is an important test quality indicator.

Test storage
Inappropriate storage conditions can affect the quality of POC tests. Countries must show evidence of assurance of test quality through a post-marketing surveillance system. Locally, it is important to establish the frequency of testing with positive and negative controls to monitor ongoing test quality and record the results as quality indicators.

Test usage
Countries should be able to show how the test is used in a national algorithm or guidelines to achieve maximum impact. In the event that different tests are used across different parts of the country, an assessment of the effectiveness of different tests should be documented.

3. Quality of testing

Staff competency
Laboratory technicians and technologists undergo training in prescribed programmes in technical colleges, national licensing bodies and certification boards. Evidence of staff training and competency at laboratories contributing data to the national database is an important quality indicator. Staff competency at adhering to existing standard operating procedures (SOPs) is also important.

Staff proficiency
In addition to general competence, staff at sentinel sites performing the tests selected for use in the elimination programme must show that they are proficient at performing the tests through external quality assessment (EQA) using a proficiency panel. EQA records for 12 months or longer are important quality indicators.

For POC tests that are performed by non-laboratory personnel, training records and evidence of good performance in EQA programmes are essential to ensure test accuracy.

lll: In-country validation mission

In-country validation (both pre-validation and validation) missions include a dedicated laboratory team made up of a team leader and one or more additional laboratory experts. The laboratory team will be asked to observe and assess the laboratory components critical for EMTCT of HIV and/or syphilis, assess how well the observed procedures represent the procedures outlined in the country report, and synthesize the information into a final report. Members of the team will visit the national reference laboratories, selected subnational laboratories (in some cases, private laboratories as well), and selected lower-level facilities.

Scope: The laboratory assessment for EMTCT is not a full laboratory audit. Rather, is an assessment of the laboratory components critical for EMTCT of HIV and/or syphilis

The assessment focuses on the four following areas:

1. **Laboratory quality management.** This is an assessment of the general organization and functioning of the national HIV/syphilis laboratory programme. In

line with existing WHO laboratory guidance, it is proposed to assess leadership and governance, including the policy framework, structure and coordination, management and supervision of the laboratory network for EMTCT. It also assesses service delivery, including organization of services, roles and responsibilities; and QC for HIV and syphilis testing among pregnant women. Other aspects assessed are supply chain management, including availability of HIV and syphilis testing materials during pregnancy, labour and delivery, and postpartum, especially if breastfeeding.

2. **Quality of tests.** This is an assessment of tests with acceptable and operational characteristics, as specified by national and international organizations such as WHO, UNICEF, the Global Fund and the USAID Waiver List or through other means (*see above*). This assessment includes areas such as the existence of national HIV and syphilis testing algorithms appropriate for prenatal testing, and choice of sufficiently well-performing tests that are appropriate for the country's clinical settings where antenatal services take place.
3. **Quality of testing**. This is an assessment of staff competency in general through professional licensure as technologists, and staff proficiency in performing the tests selected.
4. **Laboratory data management.** This is an assessment of the laboratory information management, specifically focused on a functional laboratory information system for EMTCT of HIV and syphilis.

Procedures to be followed by laboratory assessment team

Each member of the team will be asked to carry out the following steps:

Step 1. Detailed review of the country report, with special focus on HIV and syphilis testing in laboratory facilities in the network
This step is carried out prior to the mission. Specific components include:

- HIV diagnostic testing conducted for mothers and infants (antibody test for mothers and virological testing for infants), including testing done to determine mother-to-child transmission (MTCT);
- syphilis diagnostic testing for women and infants, including stillborn infants;
- evidence of proficiency and QA for HIV and syphilis testing throughout different levels of the laboratory services.

Prior to the mission, each member of the laboratory assessment team will be provided copies of the country report, national laboratory policies and plans, standards, SOPs for HIV and syphilis testing, reports from recent laboratory evaluations, and relevant documents and information on the organization and functioning of the national laboratory system. The report should include detailed information on the testing algorithms used in pregnant women and the types of HIV and syphilis laboratory tests used at various levels (e.g. national, subnational,

local); the quality of the tests used, including EQA programmes in which the country participates; country-required QA strategies, including staff training and proficiency testing programmes; and distribution of key commodities, including data on recent stock-outs. Countries will also be asked to complete self-assessments of various levels of service (national and subnational), and these should be reviewed with an eye to identifying specific laboratory challenges the country faces, which require further observation and assessment during the mission.

Important

One or more conference calls will be held prior to the mission. Any critical laboratory ***problems*** or ***omissions*** identified in the country report by laboratory assessment team members that might challenge validation should be identified during the calls. These problems must be addressed prior to the mission.

Step 2. Observation and facility assessments of in-country laboratories, with a special focus on HIV and syphilis testing

This step is carried out in country. For each mission, the laboratory assessment team will be asked to conduct on-site observations and assessments at the following:

- the national reference laboratory;
- selected regional and local laboratories, including private laboratories that conduct a substantial number of HIV and/or syphilis tests in pregnant women;
- selected lower-level (e.g. decentralized) laboratories that conduct prenatal testing, observing how laboratory practices are actually carried out.

The choice and number of laboratories to be included in the assessment will be determined prior to the mission, based on the size of the country and its laboratory network. This will be done by a regional team working with the country prior to the assessment, and members of the laboratory assessment team may be asked to weigh in on the decision. The laboratories to be visited will be chosen with an eye to understanding the country's laboratory testing system for HIV and syphilis overall, including the lowest-performing sites,[2] to ensure confidence in EMTCT. At each level, the sites to be included in the assessment should be aligned with underlying districts and facilities to allow data verification for the entire laboratory system.

Once in the country, the laboratory assessment team leader will determine the facilities to be visited by specific team members. The team is expected to communicate with each other daily about the findings.

[2] The lowest-performing subnational units can be critical subnational units in your context, where it is important to pay attention. For example, areas that could be selected as the subnational unit for review may be those with largest number of new infections, a large key population group, lowest levels of service coverage, or an estimated MTCT rate of HIV and/or congenital syphilis rate that may not meet the global EMTCT validation targets. These sites will be identified in the country report.

Important

The laboratory assessment team may identify additional laboratories or facilities that they want to add to the assessment to ensure the team's confidence in EMTCT from a laboratory perspective.

For each laboratory (e.g. national laboratory, subnational laboratory, maternity hospital, antenatal clinic, private laboratory or other facility) visited, a separate facility-specific checklist should be completed.

A separate checklist should be used for **each** facility observed, with name, date, POC test and observer information included. In facilities where testing is observed, additional checklists may be required. Each team member should fill out the appropriate checklist(s) for each laboratory they visit. These checklists will be used for the synthesis of data and results, and will also become part of the official documentation of the mission.

The checklists are intended to guide the review process to ensure that critical areas are covered, and to help adopt a common approach for all countries. Interviews will also be conducted with laboratory supervisors or managers and, as needed, laboratory technicians. Interviews with and observations of health service providers may also be needed in countries performing rapid tests or collecting dried blood spots (DBS). The main purpose of the interviews and site visits is to collect information on the checklist items, request clarification regarding information gathered from the document review, and verify information from different informants. Data are collected through direct observation, and additional information or clarification provided by the site staff. Checklists should be filled out, if possible, at the time of the interview/visit, or completed on the same day as the visit.

Note: Interviews with laboratory managers/supervisors or technicians should be scheduled by country representatives prior to the visits. It should be made clear that HIV and syphilis testing procedures may be observed during the process.

Important

Team members may be asking potentially sensitive questions about the performance or quality of work of national and local laboratory staff members. It is important that observations are made in a neutral manner that conveys clearly that the assessment will not result in any punitive action.

Step 3. Team communication on the day's observations

This step is carried out in country each day by the full laboratory assessment team. As each team member will be visiting different facilities and interviewing different staff members, no single person observes the entire laboratory system from top to bottom. Frequent communication of findings is important to better understand the system as a whole to inform future visits, and to identify challenges that should be looked into at other levels.

Daily communication should focus on overall findings with emphasis on:

- **Laboratory quality management:** sufficiently supportive leadership and governance, including policy framework, structure and coordination, management and supervision of the laboratory network for EMTCT of HIV and syphilis;

- **Quality of tests:** appropriate use of tests and sufficient availability of basic supplies, including availability of HIV and syphilis testing materials;
- **Quality of testing:** sufficient numbers of human resources of good quality, with the qualifications, training and oversight for HIV and syphilis testing. Also, sufficiently high-quality service delivery, including organization of services, roles and responsibilities, and quality control for HIV and syphilis testing among pregnant women and infants;
- **Laboratory data management:** sufficiently robust information management, including the existence of a functional laboratory information system for EMTCT of HIV and syphilis;
- **Any additional strengths/challenges** to the laboratory system that should be mentioned.

During the discussions, team members should consider specific laboratory goals for EMTCT of HIV and syphilis, such as the following:

For HIV

- Are the screening tests that are being used sufficiently sensitive to identify HIV-infected mothers/infants, including stillborn infants? (Can HIV antibody testing be performed with >99% accuracy?)
- Are the confirmatory tests/processes or algorithms able to ensure that positive women/infants are identified? Are all false positive results excluded?
- Are the QA measures in place consistent enough to ensure that staff conducting the tests are performing them correctly?
- Were there stock-outs of equipment or test kits that may have led to >4% of women/infant attendees **not** being tested and/or treated for syphilis during the past 12 months?
- Are the reporting systems for laboratory data able to support accurate counts of the numbers of women/infants tested, found to be seropositive and treated?

For syphilis

- Can the laboratories perform any NTp test with >95% accuracy?
- Are the screening tests that are being used sufficiently sensitive to identify syphilis-infected mothers/infants, including stillborn infants?
- Are confirmatory tests being used?
- Are there alternative tests to address discordant/inconclusive results and to confirm existing results as determined by comparison with another QC laboratory? (The accuracy of rapid tests for syphilis in a field setting should be ≥90%.)

Step 4. Synthesis, summary and presentation of results

This is done in country at the end of the mission, led by the laboratory assessment team leader. While synthesizing the results, some critical questions need to be answered.

(1) The team determines the adequacy of the laboratory network to provide the services needed to achieve and maintain a programme for EMTCT of HIV and syphilis in the following areas:

- Are laboratory services for diagnosing HIV and monitoring treatment universally available (i.e. for >95% of pregnant women/their infants) and accessible to all, including vulnerable and marginalized populations? (includes CD4 testing, viral load testing, qualitative PCR for infants and algorithms to assess MTCT of HIV to infants)
- Is the manner of provision of these services supportive of timely and effective diagnosis, treatment and follow up of pregnant women and exposed infants to allow EMTCT of HIV?
- Are diagnostic laboratory services for syphilis universally available (i.e. for >95% of pregnant women/their live and stillborn infants) and accessible to all, including vulnerable and marginalized populations?
- Is the manner of provision of these services supportive of sufficiently timely and effective diagnosis, treatment and follow up of pregnant women and exposed infants to allow EMTCT of syphilis? (i.e. allow effective treatment prior to 24 weeks' gestation in most circumstances)

(2) The team also determines whether the results generated by the laboratory network are reliable.

- Are there internal mechanisms to guarantee consistent application of appropriate procedures, and supervision of HIV and syphilis laboratory services?
- Are there external mechanisms for ongoing QC of laboratory services for HIV and syphilis testing for pregnant women, along with laboratory test results?

At the end of the mission, the laboratory team is expected to share findings from the assessment transparently with the participating staff and health ministry in a presentation (a few key slides) and a final report. The report should respect the anonymity and confidentiality of participating staff.

The team lead or his/her designee will be responsible for synthesizing and analyzing the data obtained through the laboratory assessments to address the findings of the overall laboratory system with regard to HIV and syphilis testing for supporting EMTCT. The presentation will have a predominantly qualitative character but will use quantitative data based on the assessment, and will address the themes assessed in **Step 3.**

Based on the joint analysis, the validation team can arrive at any of the following conclusions:

1. Unqualified endorsement of the laboratory system;
2. Endorsement of the laboratory system with clear recommendations for strengthening components that might pose a current or future threat;
3. Determination of insufficiencies that preclude EMTCT validation

The conclusions outlined in the presentation should also be summarized in a report that clearly outlines the key findings from the mission, the principal conclusions and the recommended next steps. A template for this report is provided in the Global guidance Criteria and Processes for Validation: Elimination of Mother-to-Child Transmission of HIV and Syphilis.

IV Checklists and tools used in the laboratory assessment

This section contains the checklists for laboratory self-assessment (sent prior to validation mission) for national and subnational references laboratories, **and** assessment checklists for the national validation team (NVT) and/or regional validation teams (RVT) when conducting validation missions.

Checklist 4.1 For pre-mission laboratory self-assessment:

Note: Checklist 4.1 should be sent to the laboratories being assessed prior to the validation mission. This will ensure that the laboratories are prepared and have the necessary SOPs and documents ready for review by the mission team.

This checklist can be used by the **National and Subnational reference laboratory for self-assessment.** This checklist will focus on the following 3 areas:

- laboratory quality management
- Quality of test kits used for HIV and syphilis testing
- Quality of testing

Checklist 4.2 To be used during a validation mission

This checklist will be used to confirm self-assessments conducted by National and Subnational laboratories. It will also be used by the laboratorian from the mission team conducting the laboratory assessment. Mission teams may assess the national reference laboratory, subnational or regional laboratories, including larger maternity hospitals, district hospitals, private laboratories, etc. The laboratorian who is conducting the assessment will simply check which level laboratory is being evaluated. This checklist will focus on direct observation of laboratories and the HIV and syphilis testing done, with a focus on the following areas:

- types of HIV and syphilis test kits used
- proficiency of HIV and syphilis testing done
- documentation of HIV and syphilis testing algorithms used
- availability of specialized equipment and commodities required for HIV and syphilis testing

NOTE: For each laboratory visited, the team member should fill out one or more checklist, taking care to complete the information at the top of the form.

Checklist 4.3 **To be used to assess commodities and equipment needed for HIV and syphilis testing**

Checklist 4.4 **To be used to assess and clarify which HIV and syphilis testing algorithms are used in the laboratory**

Checklist 4.5 **To be used to observe and document laboratory processes and certifications**

Algorithms for the diagnosis of HIV and syphilis

Algorithms for the diagnosis of HIV

Algorithms for the diagnosis of syphilis

Technical Recourses

Checklist 4.1 Pre validation mission self-assessment

Note: This assessment should be sent to the laboratory being evaluated so that staff has time to prepare manuals, logbooks, etc. for review by the mission team and to identify any potential problems that may interfere with validation of elimination.

☐ National level site
☐ Subnational level site
☐ Service delivery site

Name of site_____
Date of visit_____

Laboratory point person:_____

1. Laboratory quality management	Yes/No	Comments
Does a national laboratory policy exist in the country?		

Is a regulation system in place for the testing of HIV and syphilis?		
Is a national laboratory quality management system implemented?		
Is quality management and accreditation embedded in the structure of the national laboratory system?		
Does the national laboratory provide training on quality management to all public and private laboratories in the country at the central and local levels?		
Is the national laboratory equipped with a laboratory information system?		

Does the national surveillance system incorporate the laboratory information system?		
2. Quality of test	Yes/No	Comments
Is the tender process for procurement of reagents and supplies for all laboratories and testing sites managed through the Ministry of Health?		
Is there a national plan for procurement and distribution of HIV tests?		
Is there a national plan for procurement and distribution of syphilis tests?		
Is forecasting in place?		
Is there careful selection of supplies, reagents and testing kits based on:		
• Quality – sensitivity/specificity		
• Quantity – sufficient to last until next tender		

• Shelf-life – policy of "first expired, first out"		
• Ease of use		
• Conditions of use		
• Cost tendering and availability of product information?		
Is there a distribution plan allowing reagents and supplies to reach all testing sites within the appropriate time frame and prior to expiration?		
• Does the plan take into account emergency or unexpected needs?		
• Have laboratories reported stock-outs of one or more essential supplies during the previous year?		
What are the processes in place for procurement and distribution of testing materials?		
What is the policy for batch testing upon receiving diagnostic assays and the frequency of validation of these assays?		
TEST PERFORMANCE • How do HIV antibody/antigen tests perform in EQA (≥99% accuracy)?		
• How do syphilis Tp/NTp tests perform in EQA (≥95% accuracy)?		

What outside laboratories are involved in the EQA programmes?		
What are the regulations in place for storage and disposal of tests?		
3. Quality of testing	Yes/No	Comments
Does the MoH/ national reference laboratory have a means of assessing the quality of kits, reagents and supplies as they are received by the central purchasing body to ensure that standards and specifications are met?		
Does the national laboratory provide proficiency panels to regional and peripheral laboratories for HIV and syphilis?		
Does the national laboratory review the results of proficiency testing within a month?		
Does the national laboratory provide corrective action based on the results?		
Are standardized SOPs in place for HIV and syphilis testing at the central and local levels?		
Are there reliable and efficient algorithms for:		

• HIV – central- and local-level algorithms for HIV testing, CD4 count, viral load, and early infant diagnosis?		
• Syphilis – central-level algorithms and SOPs for syphilis testing?		
Is quality assurance and control procedures implemented in all laboratories across the country?		
Comment on the country's use of proficiency testing panels.		
What quality control methods are used?		
• What is the frequency of quality control at the health centres?		
• What are the average scores of the health centres for quality control testing?		
• Is there regular follow up or corrective action taken at health centres with low scores?		
• Does staff attend regular refresher training workshops?		

4.2 Checklist for assessment of the laboratory being visited during the validation mission
(COMPLETE ONE CHECKLIST AT EACH LEVEL OF GOVERNMENT ASSESSMENT)

- ☐ National level site
- ☐ Subnational level site
- ☐ Service delivery site

Name of Institution: _____

Laboratory assessed _____ Date _____

Laboratory manager _____

Contact information _____

Validation team laboratory representative _____

Laboratory assessment checklist	Information provided from self-assessment from country Please check	Verified		Comments
		Yes	No	
1. Laboratory quality management				
Does a national laboratory policy exist in the country?		Y	N	
Has the national laboratory had a quality management assessment SLIPTA validation recently?		Y	N	

Does the national reference laboratory provide the following?				
1. Supervision		Y	N	
2. Reference testing		Y	N	
3. National norms and standards		Y	N	
4. National quality assurance programmes		Y	N	
5. Licensing or accreditation?		Y	N	
Does the national laboratory provide training on quality management to the central and local levels, and private sector laboratories?		Y	N	
Is the national laboratory equipped with a laboratory information system?		Y	N	
Is the national surveillance system incorporated within the laboratory information system?		Y	N	
2. Quality of HIV and syphilis tests				
Does the laboratory participate in an external quality control programme for HIV and/or syphilis testing?		Y	N	

Ask to see last year's proficiency testing results		Y	N	
Is the tender process for procurement of reagents and supplies for all laboratories and testing sites managed through the Ministry of Health? *If No:* please see Sections 2 and 3 of subnational laboratory checklist		Y	N	
Is there a national plan for procurement and distribution of HIV tests?		Y	N	
Is there a national plan for procurement and distribution of syphilis tests?		Y	N	
Is forecasting in place?		Y	N	
Is there careful selection of supplies, reagents and testing kits based on:		Y	N	
• Quality – sensitivity/specificity		Y	N	
• Quantity – sufficient to last until next tender		Y	N	
• Shelf-life – policy of "first expired, first out"		Y	N	
• Ease of use		Y	N	
• Conditions of use		Y	N	

• Cost tendering and availability of product information?		Y	N	
Is there a distribution plan allowing reagents and supplies to reach all testing sites within the appropriate time frame and prior to expiration?		Y	N	
• Does the plan take into account emergency or unexpected needs?		Y	N	
• Have laboratories report stock-out of one or more essential supplies during previous year?		Y	N	
What are the processes in place for procurement and distribution of testing materials?		Y	N	
What is the policy for batch testing upon receipt of diagnostic assays and the frequency of validation of these assays?		Y	N	
What are the regulations in place for storage and disposal of tests?		Y	N	
3. Quality of testing				

Does the MoH/NRL have a means of assessing the quality of kits, reagents and supplies as they are received by the central procurement body, to ensure that standards and specifications are met?		Y	N	
Does the national laboratory provide proficiency panels to regional and peripheral laboratories for HIV?		Y	N	
Does the national laboratory provide proficiency panels to regional and peripheral laboratories for syphilis?		Y	N	
Does the national laboratory review the results of proficiency testing within a month?		Y	N	
Does the national laboratory provide corrective action based on the results?		Y	N	
Are standardized SOPs in place for HIV and syphilis testing at central and local levels?		Y	N	
Are there efficient algorithms for HIV and syphilis:		Y	N	

• HIV – central- and local-level algorithms and for HIV testing, CD4 count, viral load and infant diagnosis?		Y	N	
• Syphilis – central-level algorithms and SOPs for syphilis testing?		Y	N	
Is quality assurance and control procedures implemented in all laboratories across the country?		Y	N	
What quality control methods are used?		Y	N	
• What is the frequency of quality control at the health centres?		Y	N	
• What are the average scores of the health centres for quality control testing?		Y	N	
• Is there regular follow up of or corrective action taken at health centres with low scores?		Y	N	
• Does staff attend regular refresher training workshops?		Y	N	
• Are there SOPs on safety procedures and proper disposal of laboratory consumables?		Y	N	
Does the national reference laboratory participate in an external HIV quality assurance programme?		Y	N	
Does the national reference laboratory participate in an external syphilis quality		Y	N	

assurance programme?				

4. Laboratory data management				
Is there a functioning laboratory information system: any method used for documenting archived samples as long as processes for using the system are in place?		Y	N	
Are there mechanisms to ensure confidentiality of the laboratory information; are unique identifiers used for patient information?		Y	N	
How are results communicated back to the clinical site?		Y	N	
What is the average time to communicate results to the clinical site?		Y	N	
Are records archived and results easily retrievable in a timely manner?		Y	N	
Does the recording system allow for linking of HIV and syphilis test results of the mother with results of her infant?		Y	N	

4.4 Checklist for documenting HIV and syphilis testing algorithms and laboratory performance on tests

☐ National level site Name of site_____

☐ Subnational level site Date of visit_____

☐ Service delivery site

Laboratory manager _____

Contact information _____

Validation team laboratory expert _____

Algorithm (Adult)	Syphilis		HIV				EQA
	NTp	Tp	RT	EIA	IB		
Screening test							
Confirmation test							
Alternative screening test							
Alternative confirmation test							
Procedures for discordant /inconclusive tests							

Algorithm (Infant/Child)	Syphilis		HIV		
	NTp	Tp	DNA PCR	EIA	RNA PCR (viral load)
Screening test					
Confirmation test					
Alternative screening test					
Alternative confirmation test					

	Syphilis screening		Syphilis confirmation		HIVscreening		HIV confirmation		EQA
	Yes	No	Yes	No	Yes	No	Yes	No	
Appropriate patient sample									
Proficiency performed									
Controls included with each run									
Appropriate reagents (kits) used									
Reagents periodically checked[a]									
Reagent lots (kits) catalogued and monitored									
Appropriate and functioning equipment used[?*]									
Equipment in proper working condition									
Lab tools calibrated[a]									
Manufacturer's protocols followed									

4.5 Checklist for observing and documenting laboratory processes and certifications

- ☐ National level site
- ☐ Subnational level site
- ☐ Service delivery site
- ☐

Name of Institution: _____

Date _____

Laboratory manager _____

Contact information _____

Validation team laboratory representative _____

General laboratory observations	Yes	No	N/A	Comments
Does the laboratory have ISO 15189 certification? Check most recent certification certificate. If ISO 15189 status is current, may check non-availability of basic laboratory supplies and procedures as these are guaranteed to be in place if ISO 15189 designated	Y	N		
Participation in an external QC programmer. Which? Review quarterly QC reports covering 3 years.	Y	N		
Participation in either SLIPTA or SLMTA programme Review status	Y	N		
Source of deionized (DI) water				
Adequate electricity	Y	N		
Back-up for electrical system	Y	N		

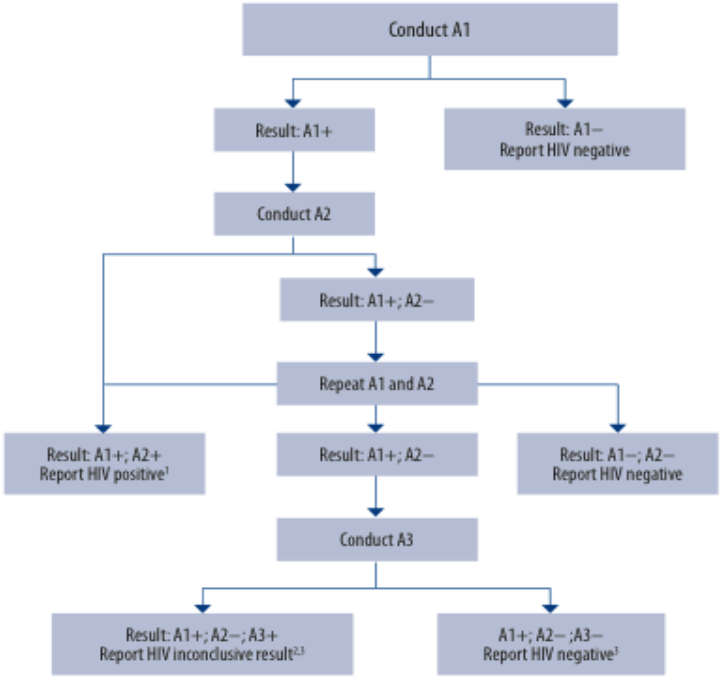
Calibration/maintenance of essential equipment	Y	N		
Equipment functionality monitored	Y	N		
Logbooks for equipment records	Y	N		
Logbooks for specimen records	Y	N		
Logbooks for reagent records	Y	N		
Records on computer	Y	N		
Computer back-up	Y	N		
Protocol logbook	Y	N		

Protocols for corrective actions	Y	N		
Specimen collection and handling				
Proper storage of test kits and reagents	Y	N		
Proper equipment for processing sera, plasma, cells	Y	N		
Proper storage of patient specimens (including dried blood spots [DBS])	Y	N		
Report on stock-outs of testing supplies over the past 12 months (stock-out is defined as lack of at least one basic supply for >2weeks)	Y	N		
Does the laboratory have a back-up plan for sample storage during stock-outs? (i.e. freeze and test when supplies become available?)	Y	N		

Algorithm for HIV testing

Source: Consolidated guidelines on HIV testing services. Geneva: World Health Organization; 2015.

Figure 1. HIV testing strategy for diagnosis in high prevalence settings



Notes:
"Assay A1", "A2", "A3" represent three different assays (of any test format). "Report" = result may be reported.
1 For newly diagnosed individuals, a positive result should be confirmed on a second specimen to rule out laboratory error.
2 Re-testing should be performed on a second specimen taken after 14 days to rule out seroconversion.
3 If A1 is an antigen/antibody detection assay and A2 or A3 is an antibody-detection-only assay, re-testing should be performed with a second specimen taken after 14 days.

serological assays. It would require adaptation if NAT technologies were used as A2 or A3.

All specimens are first tested with one assay (A1), and specimens that are non-reactive (A1–) are considered HIV-negative and reported as such. A1 should be the most sensitive assay available, taking into account diagnostic sensitivity, seroconversion sensitivity and analytical sensitivity.

Any specimens that are reactive on the first assay (A1+) should be reflexed (tested again) using a separate and distinct second assay (A2) comprising a different antigen preparation to avoid false cross-reactivity with A1. For specimens that are reactive both

Fig. 7.3. Testing strategy for HIV diagnosis in high prevalence settings

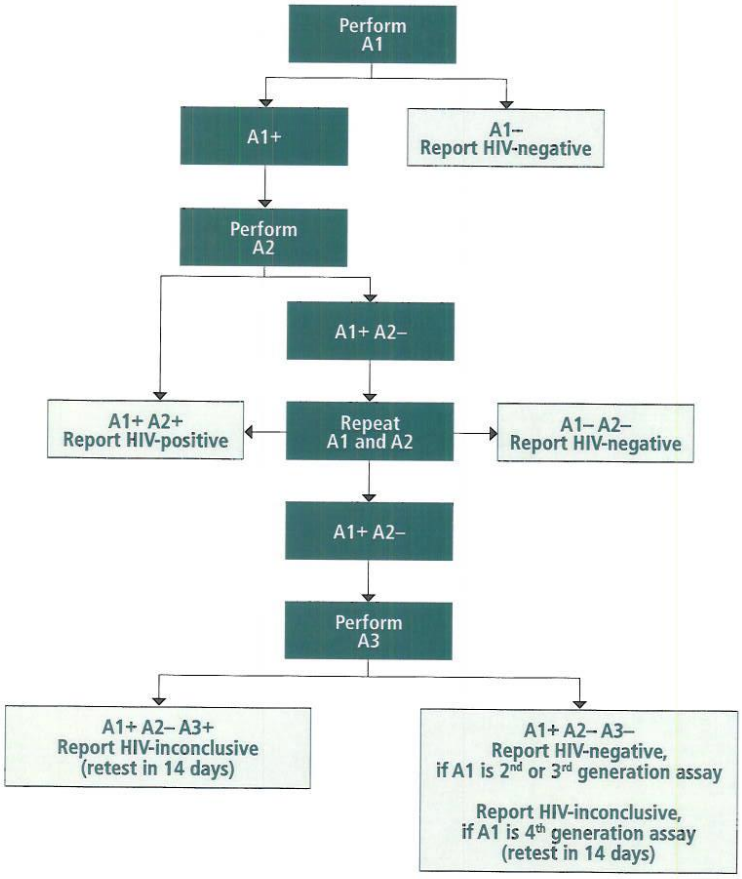
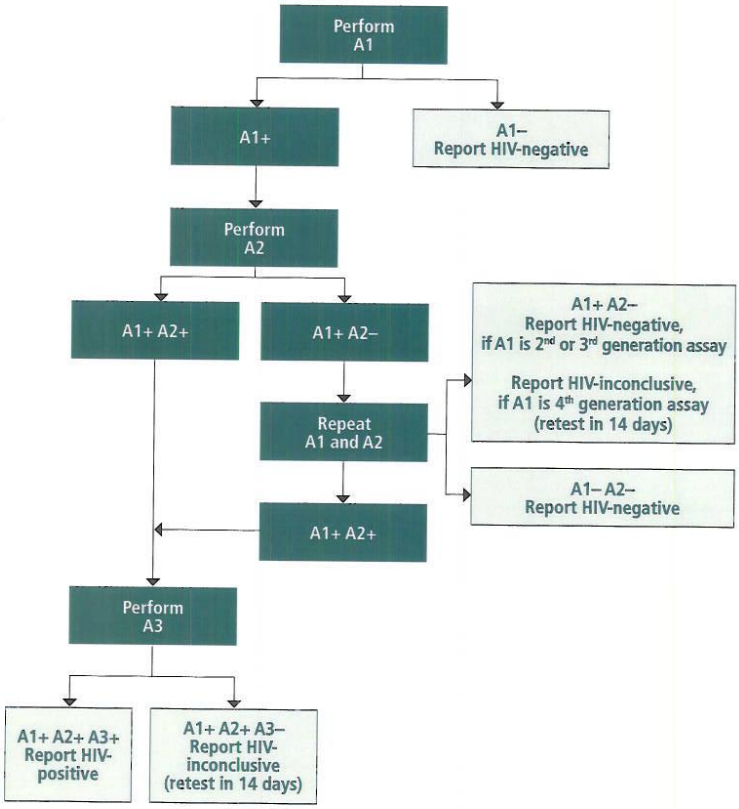


Fig. 7.4. Testing strategy for HIV diagnosis in low prevalence settings



All specimens are first tested with one assay (A1), and specimens that are non-reactive (A1–) are considered HIV-negative and reported as such. A1 should be the most sensitive assay available, taking into account diagnostic sensitivity, seroconversion sensitivity and analytical sensitivity.

Any specimens that are reactive on the first-line assay (A1+) should be retested using a separate and distinct second assay (A2) comprising a different antigen preparation to avoid false cross-reactivity with A1.

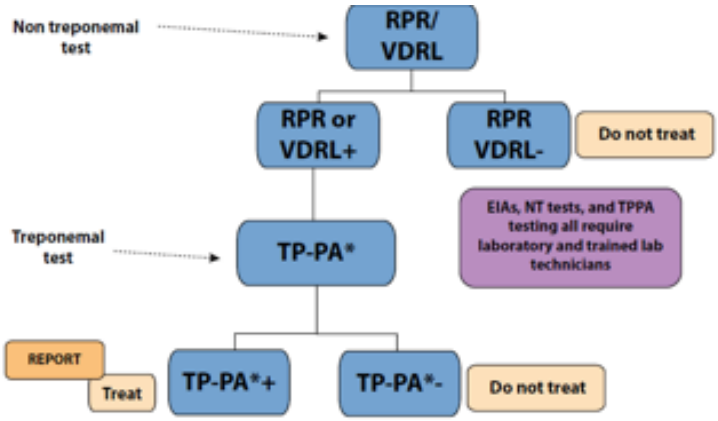
Specimens that are reactive on the first-line assay but nonreactive on the second-line assay (A1+; A2–) should be repeated using the same specimen with the same two

Algorithms for the diagnosis of syphilis

Source: Guidance on syphilis testing in Latin America and the Caribbean: improving uptake, interpretation, and quality of testing in different clinical settings. Washington, DC: Pan American Health Organization; 2015.

The following are examples of algorithms that might be used by laboratories or clinical settings conducting syphilis testing.

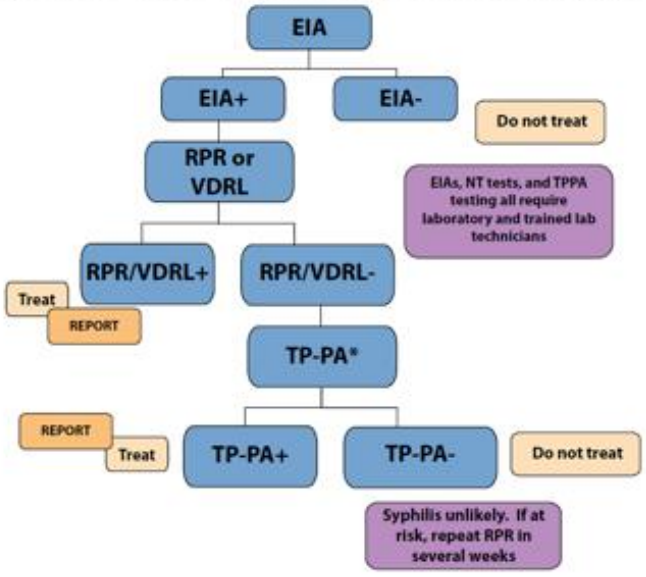
Figure 1. Traditional laboratory-based syphilis serologic testing
Non-treponemal screening test confirmed with treponemal test



- Approach detects active infection and can be used for routine surveillance reporting.
- RPR test has a high rate of biologic false positive, but when used with confirmatory treponemal test (TPPA) has a high positive predictive value
- This algorithm may miss early primary or old, past infection
- Requires a laboratory with trained technicians and appropriate equipment
- Routine reporting of cases for surveillance

*Other treponemal tests could be used; however, TP-PA has better performance than TPHA and FTA-ABS, and is less expensive than FTA-ABS

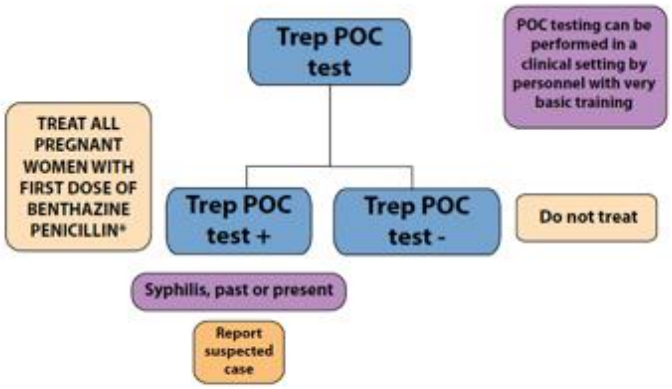
Figure 2. Reverse sequence laboratory-based syphilis serologic testing
Treponemal screening test to allow automated screening, confirmed with non-treponemal test



- Approach detects early primary or past infection that might be missed with initial RPR testing. The non-treponemal test detects active infection.
- EIAs and CIAs have imperfect specificity, i.e., a high rate of false positive results, and results dependent upon risk of the population tested. Positive results should be confirmed with a TP-PA
- Confirmatory treponemal test must have equivalent sensitivity but greater specificity than the screening EIA
- Requires a laboratory with trained technicians and appropriate equipment
- Approach can be automated (fewer human resources) but can be expensive and time consuming
- This algorithm can be used for routine reporting of cases for surveillance (includes confirmatory test)

*Other treponemal tests can be used; however, TP-PA has better performance than TPHA and FTA-ABS, and is less expensive than FTA-ABS

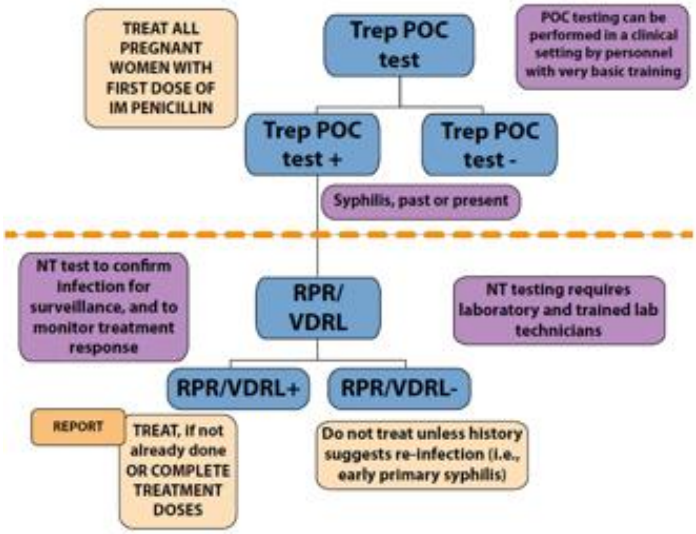
Figure 3. Treponemal rapid point-of-care (POC) syphilis testing – without confirmatory non-treponemal test
Clinic-based testing opportunity best used in antenatal clinic setting



- Point-of-care test (POC), such as a single rapid treponemal test or combined HIV/syphilis rapid test, can be performed at the site of the visit (health center), facilitating same-visit testing and treatment and minimizing loss to follow up
- Blood can be drawn for a laboratory-based treponemal test (RPR or VDRL) to confirm the presence of active disease, clarify need for further treatment of mother and partner management, and to monitor treatment response
- Cost effective for the prevention of congenital syphilis
- Reporting to surveillance should indicate test type used (i.e, POC with no confirmation). If laboratory based non-treponemal test is later performed, the initial case report should be updated.

*Treatment benefits for infant is greater than risk of maternal treatment

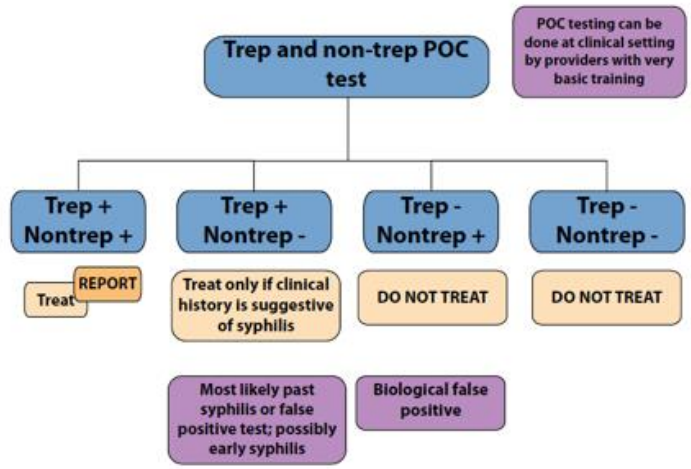
Figure 4. Treponemal rapid point-of-care (POC) syphilis testing – with confirmatory non-treponemal (NT) testing
Clinic-based testing opportunity, consider in settings serving patients who may have been previously treated for syphilis



- Addition of non-treponemal test identifies active infection, reduces over-treatment, facilitates same-day testing and treatment (minimizing loss to follow up), and provides better surveillance data
- POCT can be performed at site of visit (health center), while RPR or VDRL requires laboratory
- This algorithm can be used for routine surveillance reporting (includes confirmatory test)

*Treatment benefits for infant is greater than risk of maternal treatment

Figure 5. Dual non-treponemal/treponemal (NT/T) antigen rapid point-of-care (POC) syphilis testing
Clinic-based testing opportunity



- Identification of active, confirmed infection at site of clinic visit (health center)
- Does not require laboratory
- Facilitates same-day testing and treatment, minimizes loss to follow up
- This algorithm can be used for routine reporting, as confirmatory testing is part of POC

EIA enzyme immunoassay, FTA-ABS fluorescent treponemal antibody-absorption (test), NTp/Nontrep non-treponemal, POC point of care (test), RPR rapid plasma regain, TP-PA *Treponema pallidum* particle agglutination, Tp/Trep treponemal, TPHA *Treponema pallidum* haemagglutination assay, VDRL Venereal Disease Research Laboratory (test)

Summary of proper procedures and common sources of error during HIV and syphilis testing

General laboratory measures

Background

Many activities outside of actual testing are required to maintain QC in a clinical laboratory. These include the maintenance and routine calibration of many items of equipment, and monitoring of environmental factors, including water quality and temperature. Variations in these basic conditions and needs can greatly affect the outcome of test results.

Proper procedures

General laboratory measures

1. The laboratory should be continually monitored for room temperature, water quality and equipment status.

2. Refrigerators and freezers should be properly maintained and their temperatures recorded daily.

3. Centrifuges need to be monitored and calibrated at least once a year.

4. Pipettors and other bench equipment (e.g. thermometers) need to be calibrated at least once a year. Those that cannot be adjusted need to be replaced. Accurate and sterile handling of patient samples and reagents is critical to the validity of all test results.

5. Some equipment such as rotators need to be monitored and, if necessary, calibrated, at least on a weekly basis.

Reagents

1. Chemicals and laboratory reagents should be of high quality. The dates that a chemical is received and/or a reagent is prepared should be marked on the container. Outdated chemicals and/or reagents should be discarded and replaced.

2. Reagents made in a laboratory need to be checked immediately after preparation to be sure that they maintain the same quality as previous reagents.

3. The source of the laboratory's distilled water supply should be monitored weekly to be certain that reagents such as buffers, diluents, etc. meet the specifications required by the tests.

4. Commercial test kits should also be monitored for their expiration dates. Expired kits should be discarded.

5. Some kits/reagents should be routinely tested with both positive and negative samples to ensure that their quality has not deteriorated (qualitatively and quantitatively).

Sources of error

1. Samples, tests, reagents, etc. are exposed to/performed at incorrect temperatures.
2. The laboratory's distilled water source is not suitable (contains mineral salts).
3. A critical piece of equipment is out of calibration (centrifuge, rotator, pipettor, etc.).
4. A critical reagent or test kit is out of date or has deteriorated.

Specimen collection and handling

Background

Specimen collection and handling is a critical part of any laboratory testing procedure. Improperly collected (e.g. haemolysed or contaminated blood) or stored specimens will greatly affect the test results. Each type of patient specimen (serum, plasma or cerebrospinal fluid [CSF]) has a different shelf-life and storage requirement. Some may be tested within 5 days (serum); others may need to be tested within 48 hours (plasma).

Proper procedures

1. Venous blood should be collected in a vacuum tube using an 18- or 21-gauge needle. (Smaller needles can be used for infants and children.)
2. Proper safety precautions (wearing personal protective equipment) should be exercised during extraction and processing of blood.
3. For serum collection, blood should be allowed to clot for at least 30 minutes at room temperature before making any attempt to collect it. The tube with the blood clot should be centrifuged for 10–15 minutes at 1000–1200x *g* and the serum immediately removed. Serum can be stored between 2°C and 8°C for up to 5 days for testing purposes. If longer storage is required, it should be frozen at –20°C. Repeated thawing and freezing should be avoided.
4. Plasma collection is identical to that of serum, except that anticoagulants are in the tube to prevent clotting of blood. Centrifugation should be performed within minutes after collection. Plasma must be used within 48 hours for syphilis testing, during which time it needs to be stored between 2°C and 8°C, if testing is delayed by more than 4 hours. Plasma specimens cannot be heated.
5. CSF should be collected by lumbar puncture. It should be free of traces of blood. It should be treated in a manner identical to that for plasma with respect to testing and storage.
6. DBS should be collected from a finger-stick onto Whatman 903 filter paper cards and the blood allowed to air-dry for a minimum of 4 hours. The specimens should be stored in a zip-lock bag with desiccant at –20°C. One 6 mm punch should be removed from the DBS and eluted with 150 µl of phosphate-buffered saline (PBS) containing 0.05% Tween 80 at 4°C either for a minimum of 4 hours or overnight. DBS and dried blood tubes (DBT) are acceptable for HIV, and for syphilis Tp tests only. Their reliability has not been sufficiently evaluated for NTp tests to diagnose syphilis. In fact, many studies indicate serious problems using DBS extracts for NTp tests.
7. For each specimen tested in the laboratory, the lot number of the reagents used and/or the test kits should be recorded for future reference.

Sources of error

1.Improper collection may result in haemolysis or contamination of blood, and/or the presence of excessive particulate matter in the collected specimen

2. The conditions for storage of patient specimens may be incorrect.

3. Patient specimens may be improperly labelled and/or tracked.

4. Use of DBS or DBT samples for NTp syphilis tests can lead to erroneous results.

Enzyme-linked immunoassay (ELISA/EIA)

Background

The ELISA or EIA is an enzymatic immunoassay to detect the presence of HIV and treponemal antibodies. There are four generations of HIV ELISAs. The first generation used viral lysate to detect human IgG antibodies against HIV antigens. The second-generation ELISA used recombinant viral protein (p24) to detect human IgG antibodies against HIV antigens. The third-generation ELISA used HIV antigens in a "sandwich" method to detect both human IgG and IgM antibodies against HIV antigens. This generation of tests can usually detect seroconversion a week earlier than the second-generation tests. The fourth-generation ELISA uses HIV antigens and p24 antibody in a "sandwich" method to detect both human IgG and IgM antibodies against HIV antigens, and the presence of p24 viral protein. The fourth generation can usually detect seroconversion 1–2 weeks earlier than the third-generation tests. For the diagnosis of both HIV and syphilis, there are numerous variations of this assay and many commercial kits are available. Each kit has its own specific instructions, which should be followed to the letter. Even though they work in a similar fashion, they can be divided into basically two different types of assays. One type uses an anti-human IgG or IgM conjugate; and the other uses an antigen conjugate ("sandwich"). Generally, the latter is more specific, and has a lower false-positivity rate as well as greater sensitivity The only major difference in the various ELISAs is the antigens immobilized in the wells to capture human antibody.

Brief procedure

1. According to the manufacturer's specifications, specimens should be diluted and placed in microtitre plate wells for testing. (Kit controls should be included.)
2. The microtitre plate should be incubated for a specified amount of time as per the conditions specified in the kit instructions.
3. All wells should be rinsed with PBS or other solution specified by the manufacturer. This usually requires at least three separate rinse and aspiration cycles.
4. The appropriate conjugate should be added to each of the wells and the plates incubated according to the kit instructions.
5. Step #3 is then repeated.
6. The substrate mix should be prepared according to the kit's instructions and dispensed into each of the wells of the microtitre plate. This should be a timed reaction.
7. At the specified time, the enzymatic reactions are terminated with a stop reagent (typically concentrated H_2SO_4).

8. The plates should be read (usually within 30 minutes) using the manufacturer's guidelines at a specific wavelength tailored for the substrate.
9. Test results: manufacturers typically give a cut-off point, or a method for calculating the cut-off point for their particular assay. Any absorbance value above the cut-off point is considered positive and any value below the cut-off point is considered negative. There may be a range around the cut-off point that is deemed weakly positive.

Sources of error

1. Contaminated, haemolysed or lipaemic serum samples can result in false-positive results.
2. Kits have expired or have not been stored properly.
3. Reagents have not been warmed to room temperature.
4. Plates have not been properly rinsed between each step.
5. The enzymatic reactions have not been properly timed and have not been stopped as per the manufacturer's instructions.

Footnote

The quality assurance of EIAs should apply to testing for both syphilis and HIV. The primary difference is the antigens used in the specific tests.

Immunoblotting assays

Background

Blotting tests are immunological assays performed using nitrocellulose strips containing immobilized treponemal antigens. For HIV infection, antibodies against the following antigens are detected: gp41, gp120/gp160 from the viral envelope; p17, p24 or p55 from the virus core; and p31, p51 and p66 viral enzymes. For a person to be considered HIV positive, they need to have antibodies against one envelope and one core protein or against one of the enzymes. To diagnose syphilis, usually three or four immobilized antigens are attached to the strip. The most common antigens are the 15 kDa, 17 kDa, 38 kDa and 47 kDa treponemal antigens. A few kits have become commercially available.

Brief procedure

1. The patient's serum is incubated with the immunochromatographic strip for a specified amount of time based on the manufacturer's instructions.
2. The strips are washed with buffer usually containing some type of detergent to remove non-specific antibody binding.
3. The chromatographic strips are then incubated with an anti-human enzyme conjugate for a specified amount of time. Anti-human antibodies against either or both IgG and IgM can be used.
4. Step two is repeated, and then a chromatographic mix is incubated with the strips to visualize the presence of human antibody.
5. Test results: at least two of the antigen strips on the immunochromatographic strip should be labelled with human antibody in order for the test to be deemed positive (see

manufacturer's insert). The intensity of each band should meet the manufacturer's specifications. (Usually a visual aid is included in the kit.)

Sources of error

1. Kits are outdated.
2. The kits have been improperly stored.
3. Too much patient sample has been used.
4. The strips have not been rinsed properly.
5. The test results may be "overread". (A faint or partially coloured line is many times considered negative; see the manufacturer's instructions.)
6. The control line does not change colour (bad strip).

Footnote

The quality assurance of immunoblotting assays should apply to testing for both syphilis and HIV. The primary difference is the antigens used in the specific tests.

Venereal Disease Research Laboratory test

Background

The VDRL is a microscopic flocculation test to determine the presence of antibodies against cardiolipin (reagin). A microscope is required.

Brief procedure

1. Fresh VDRL antigen must be prepared each day and should be used within 2 hours of preparation.
2. 50 µL of either serum or CSF is placed in a paraffin ring on a slide using a pipette.
3. Approximately 17 µL (one free-falling drop from a dispensing needle) of the VDRL antigen is added to the patient sample.
4. The specimens are placed on a mechanical rotator for 4 minutes at 180 rpm. (A humidifying cover should be placed over the slide to prevent evaporation.)
5. After 4 minutes, the slide should be examined using a microscope and read using a 10X objective.
6. Test results: the presence of medium or large clumps indicates that the test is reactive; small clumps mean the test is weakly reactive; and no clumps or slight roughness indicate that the test is non-reactive.
7. Twofold serial dilutions of the patient sample can be prepared to determine a quantitative end-point.
8. When using CSF, 10 µL (or one drop from a 21- or 22-gauge needle) of the VDRL antigen is added to the patient sample, and is incubated for 8 minutes instead of 4 minutes.

Sources of error

1. Prozone reaction: the antibody titre is so high that flocculation is inhibited or does not occur.

2. The VDRL antigen is not freshly prepared.
3. Plasma may have been used, which is not an acceptable patient specimen.
4. Biological false-positive reactions can occur. The VDRL must be linked to other tests.
5. Temperature variation may occur. Temperatures out of the 23–29°C range can cause a significant increase in false-positive test results.
6. The mechanical rotor has not been routinely calibrated (180 rpm ± 2).
7. The microscope objective is dirty or the lens quality has deteriorated.
8. NTp tests will be positive in regions where yaws is endemic.
9. In persons treated in the latent or late stages of syphilis or those who have become reinfected, the titre of the quantitative test does not decrease as rapidly as among those with primary syphilis.
10. DBS or DBT cannot be used as specimens.

Qualitative rapid plasma reagin test

Background

The RPR is a flocculation test used to determine the presence of human antibodies against cardiolipin (reagin). The results are read visually and a microscope is not required. Only qualitative RPR is described here as quantitative RPR is generally reserved for monitoring treatment.

Brief procedure

1. Fresh RPR antigen is prepared and can be used for several days if properly stored. Validation of the antigen should be performed on each day that it is used for testing.
2. Validation consists of a negative reaction with non-reactive serum and a positive reaction with reactive serum. When new lots are prepared, they must be quantitatively compared with the previous lot to ensure their efficacy.
3. 50 µL of either serum or plasma is placed in a paraffin ring on an RPR card using a pipette. The sample should be spread out to cover the entire 18 mm circle.
4. Twofold serial dilutions of the patient sample from 1:2 to 1:16 are prepared to determine a quantitative end-point.
5. Approximately 17 µL (one free-falling drop from a dispensing needle) of the RPR antigen is added to the patient sample.
6. The specimens are placed on a mechanical rotator for 8 minutes at 100 rpm. (A humidifying cover should be placed over the slide to prevent evaporation.)
7. After 8 minutes, the card should be tilted and slightly rotated, and clumping or roughness noted. A comparison image reference (visual aid) is useful.
8. The test reactions are read under high-intensity incandescent lamps.
9. Test results: intense-to-moderate clumping indicates that the test is reactive; slight clumping indicates a weakly reactive result; and no clumps or slight roughness indicates a non-reactive result.

Sources of error

1. Prozone reaction: the antibody titre is so high that flocculation is inhibited or does not occur.

2. The RPR antigen is not qualitatively monitored. (Preparations are usually good for a maximum of 2 weeks. As the quality of the reagent deteriorates, its reactivity diminishes.)
3. Biological false-positive reactions can occur. RPR must be conducted in conjunction with other tests.
4. CSF is not an acceptable patient specimen for the RPR.
5. Temperature variation: temperatures out of a range of 23–29°C can cause a significant increase in false-positive test results.
6. The mechanical rotor has not been routinely calibrated (100 rpm ± 2).
7. There is inadequate lighting.
8. The antigen has been improperly spread within the circle or has leaked outside the circle.
9. NTp tests will be positive in regions where yaws is endemic.
10. DBS or DBT cannot be used as specimens.

Fluorescent treponemal antibody-absorption test

Background

The fluorescent treponemal antibody-absorption (FTA-ABS) test is a fluorescence microscopy technique to determine the presence of treponemal antibodies in patient serum. A microscope is required. It was once considered the "gold standard" test for diagnosing syphilis.

Brief procedure

1. Pre-prepared slides with fixed treponemes should be used. (In-house preparation of slides otherwise requires: (i) a source of organisms (from rabbits), and (ii) significant additional measures of quality control to ensure that the test results are accurate.)
2. Patient serum must be pre-absorbed to reduce or minimize background reactivity. Special precautions must be taken to ensure its quality.
3. 200 µL of sorbent is added to a tube with 50 µL of heated patient serum, mixed, and 30 µL of that mix is placed on the pre-prepared slides. (+1 to +4 controls also run.)
4. The specimens are incubated for 30 minutes at 35–37˚C. (A humidifying cover should be placed over the slide to prevent evaporation.)
5. After 30 minutes, the slides should be rinsed with PBS and then allowed to set in a dish of PBS for 5 minutes and then rinsed again. (The rinsing procedure is critical.)
6. 30 µL of diluted fluorescein isothiocyanate (FITC)-labelled anti-human IgG in PBS with Tween 80 is placed on each smear. Repeat steps 4 and 5. Step 5 should be carried out in the dark.
7. After the slides have been blotted dry, mounting medium should be added and the slides observed within 4 hours. (Slides should be kept in the dark until read.)
8. Test results: the patient's samples are compared with the internal controls. A +2 to +4 intensity is reactive; +1 is minimally reactive; and <+1 is non-reactive.
9. For a specific patient sample, several areas of the slide should be examined for consistency.

Sources of error

1. The quality of the absorbent and FITC antibody reagent is critical. (Some of this concern has been eliminated by the commercial availability of kits.) However, their quality must be monitored, especially if the test is not routinely run.
2. If the reagents become contaminated or have not been stored properly, the results will probably be erroneous.
3. If multicircle slides are used, precaution must be taken to ensure that the patient samples do not cross-contaminate one another.
4. If the rinsing procedures are not properly followed, the background fluorescence may be high and make the results difficult to read and interpret.
5. The physical condition and maintenance of the fluorescence microscope is critical.
6. Much error is introduced by subjective reading of slides. Many technicians label any fluorescence visible as a positive result. Careful comparison with the standards is required.

Treponema pallidum particle agglutination assay and *Treponema pallidum* haemagglutination assay

Background

The *Treponema pallidum* particle agglutination assay (TP-PA) is widely used. The major difference between the TP-PA and the microhaemagglutination assay (MHA)-TP is the nature of the particles used in the agglutination. MHA-TP uses sensitized red blood cells, and the TP-PA uses sensitize gel particles. The test procedure for the *Treponema pallidum* haemagglutination assay (TPHA) is the same, with slightly different antigens.

Brief procedure

1. Most reagents come pre-prepared in a commercially available kit.
2. 100 µL of sample diluent is placed in the first well of a U-bottomed microtitre plate and 25 µL is added to the second, third, fourth, and succeeding wells. Ten wells are used for each patient specimen.
3. 25 µL of patient sample is added to the first well and mixed; 25 µL of this mix is transferred to the second well. Twofold serial dilutions are continued until all 10 wells have been filled. A reactive and non-reactive control should also be included with each run.
4. 25 µL of reconstituted sensitized particles is added to each of the wells.
5. The microtitre plates are placed on an automatic vibratory shaker for 30 seconds.
6. The plates are then covered and incubated for 2 hours at room temperature or overnight.
7. The settling patterns are read using an angled mirror (tray viewer) to visualize them. Use of a visual aid reference is advised.
8. Test results: agglutinated particles covering the bottom of the wells uniformly or a definite large ring with a rough multiform outer margin with peripheral agglutination is considered reactive; particles concentrated in the shape of a compact ring with a smooth round, outer margin is considered weakly reactive; and particles concentrated in the shape of a button in the centre of the well is considered non-reactive.

Sources of error

1. The microtitre plates must be U-bottomed, not flat, and not V-shaped.
2. The tray should be incubated in a stable, vibration-free environment.
3. The trays may not have been properly covered, especially when incubating overnight.
4. There may be cross-contamination of the microtitre well.
5. The kits may not have been properly stored.
6. The results of the tests may be misread (reactive vs intermediate).

Rapid point-of-care tests

Background

Rapid tests (RTs) are immunological assays run on chromatographic strips of nitrocellulose. They offer the advantage of a very short incubation time and quick test results. As the patient may be tested and treated at the same visit, they are often called point-of-care (POC) tests. Like the ELISA tests for HIV diagnosis, there are four generations of rapid tests for HIV detection. Only the fourth-generation RTs will detect both human antibody and p24 HIV antigen. Although the most widely available RTs for diagnosing syphilis are treponemal, recently dual RTs have been developed, which can test for both treponemal antibodies as well as those against cardiolipin (non-treponemal). In addition, new combination RTs (such as HIV/treponemal, and HIV/treponemal/hepatitis B, for example) are also being developed.

Brief procedure

1. Procedures vary according to the manufacturer's specifications.
2. Routinely, 5–10 μL of patient blood is obtained via a finger-stick, and applied to the sample pad on the RT kit. Some kits can also use serum or CSF.
3. After application, the sample is chased with a specified amount of buffer. Some kits have a single buffer application and some have two buffer applications.
4. Test results: after a specified amount of time, the results are visualized as a line containing immobilized antigen changing colours, usually from white to red. A control line should also turn red in all test cases or the test is invalid.
5. In most cases, the reading of the test should occur within 5–15 minutes after the reaction is complete (specified by the manufacturer).

Sources of error

1. The kits may be outdated.
2. The kits may have been improperly stored.
3. Too much patient sample has been used.
4. The test results may have been "overread" (a faint or partially coloured line is often considered negative).
5. The control line does not change colour (bad test kit).

Footnote: it is recognized that the use of RT-POCs requires far less surveillance of the QC of reagents, facilities and the personnel who perform the tests than conventional laboratory testing. However, RTs from different companies and even different lots from a single

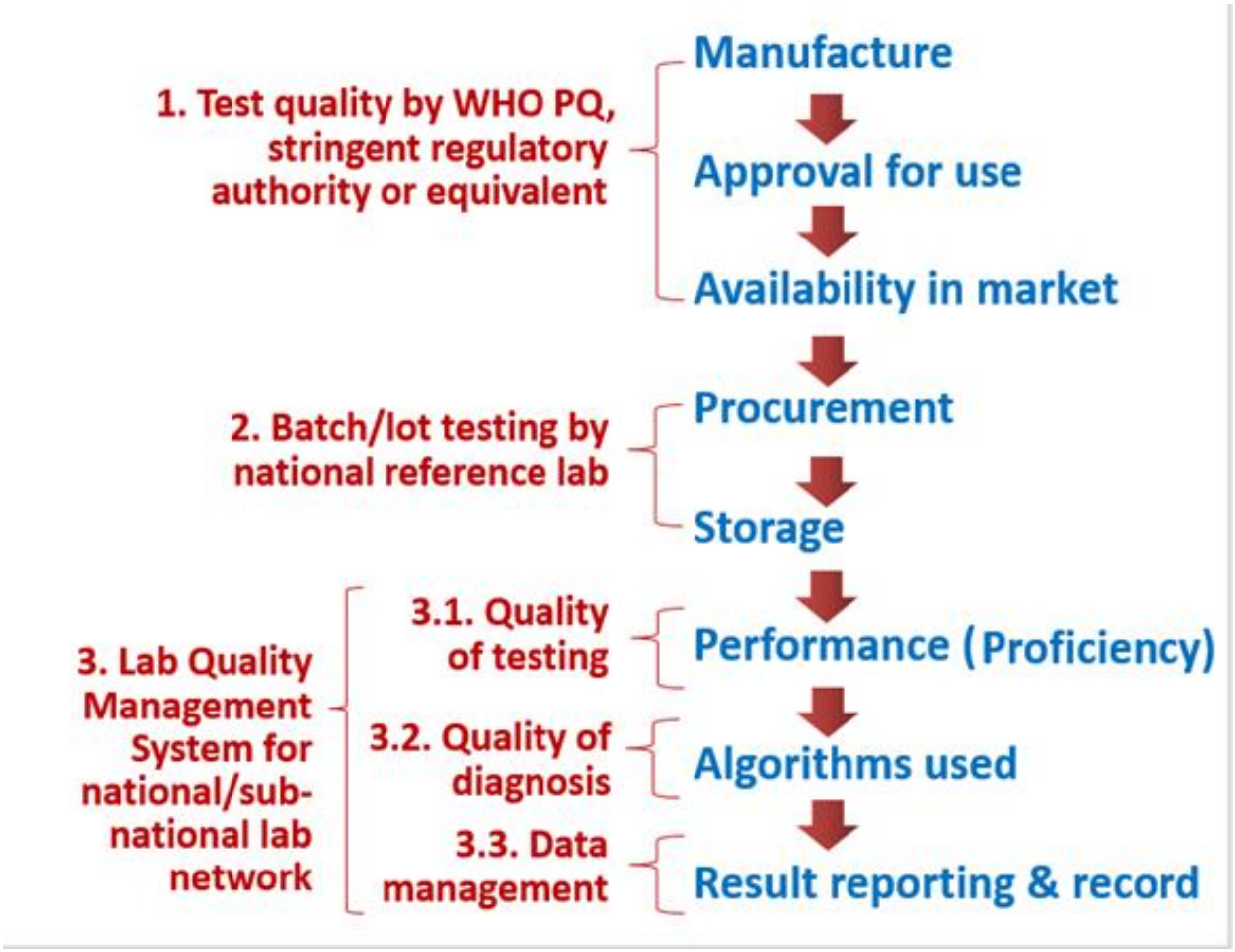
company may vary. In addition, the accuracy of RT results at field sites is generally 5–10% less than those in a laboratory setting. Even with fewer reagents and less physical "overhead", it can still be a challenge to maintain QC and test the accuracy of RTs. Even though most require only 1–4 steps to perform, personnel training and oversight should not be taken for granted, and should be routinely checked and monitored. In addition, it is recommended that when a new RT is introduced or a new lot of RTs is introduced, QC testing should be performed. If a new test is introduced, at least 50 devices (25 with positive sera and 25 negative) from each manufacturer should be tested with the same serum and the results compared. If a new lot of the same test is being introduced, at least 5 positive and 5 negative sera from each lot should be compared. These measures will ensure continuity in field-testing. The QA of RTs should apply to those for both HIV and syphilis.

Quality indicators to assess the veracity of laboratory data

To assist countries with assessment of their progress towards disease elimination, it is critical that countries examine their laboratory infrastructure for the existence of quality indicators of laboratory data produced at different levels of the health system. The accuracy and reliability of laboratory data that contribute to the evidence of disease elimination cannot be ascertained without these quality indicators.

1) *Laboratory quality management:* progress towards ISO 15189 as specified by the International Organization for Standardization (ISO);
2) *Quality of tests:* based on acceptable performance and operational characteristics as specified by national and international organizations such as WHO, UNICEF, the Global Fund, and the USAID Waiver List;
3) *Quality of testing:* staff competency in general through professional licensure as technologists, and staff proficiency at performing the tests selected above as shown through EQA or proficiency panel testing. See fig 1

Fig 1



1. Laboratory quality management

The assessment of laboratory data in a country can start with whether a national laboratory policy has been developed and implemented. Quality management of laboratories should be defined within the national laboratory policy document, including how laboratory quality is monitored.

Accreditation as a quality indicator

A laboratory is accredited by a reputable accreditation body if the laboratory has attained a prescribed level of technical competence to perform specific measurements, and produces data that are accurate, traceable and reliable.

The ISO has established criteria for the quality management of laboratories as specified in ISO 15189. While attainment of ISO 15189 is an aspiration of many laboratories, it is helpful for countries to measure progress towards accreditation through a step-wise process. The WHO Regional Office for Africa has established a step-wise framework to encourage, support, and recognize the implementation of quality management systems in medical laboratories in Africa. The SLIPTA (Stepwise Laboratory Quality Improvement Towards Accreditation)

Programme awards stars as laboratories achieve more than 55% of items on the SLIPTA checklist as scored by evaluators from the African Society for Laboratory Medicine (ASLM) (Fig. 2).

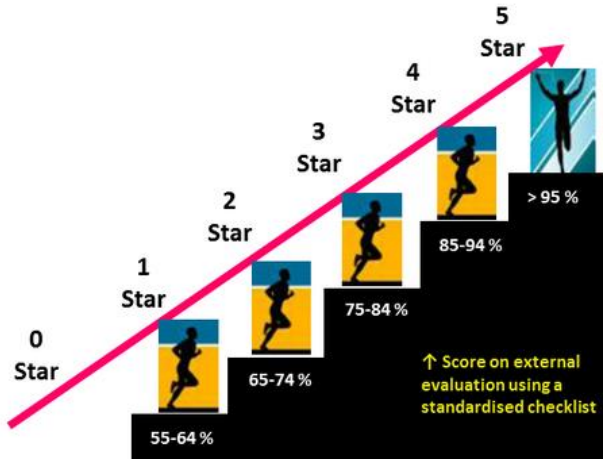


Fig. 2: The WHO Stepwise Laboratory Quality Improvement Towards Accreditation

Similar laboratory quality management programmes may exist in different regions of WHO, and it is useful for countries to show how laboratories in the country are organized and managed in the context of the HIV and syphilis control programmes, as countries make progress towards elimination.

Organization of laboratories contributing data towards elimination

In developing countries, laboratory infrastructure may exist only for major disease control programmes such as HIV, tuberculosis and malaria, and is often less robust or non-existent for many neglected diseases, including syphilis. This makes the collection of data for national statistics on such diseases difficult, often patchy and unreliable.

In the absence of a system of local, district and regional laboratories contributing to a national database, it is possible for countries to set up disease-specific sentinel sites to collect data that are representative of national trends or progress towards elimination (Fig. 3). The number and distribution of sentinel sites depends on the laboratory infrastructure in place, the geographical distribution and epidemiology of the disease in question, and must give a representative picture of disease incidence across the country.

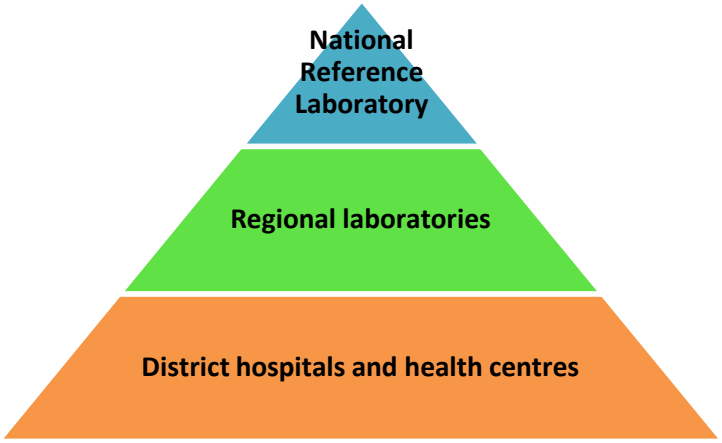


Fig. 3: Laboratory infrastructure at different levels of a health-care system

Laboratory data management

Verification of data management practices at the national level, different levels of the laboratory system and at sentinel sites across the country, and of the data transmission mechanisms to a central database is critical to ensuring the quality of data that demonstrates that a country has reached elimination targets.

Accreditation requirements for hospitals at different levels of the health system

Laboratory	Optimal	Minimum
National level	ISO accreditation (ISO 13485) LIMS or LIS connected to the MoH health information system Organize national EQA system for all lower-level laboratories	Participate in SLIPTA and SLMTA or equivalent Organize EQA for all sentinel surveillance sites
Regional level	LIS	
District level	LIS	
Sentinel sites	LIS	Standardized forms available to collect surveillance data