Syphilis Testing Practices in the Region of the Americas: Results of the 2014 Survey

CDC
Centers for Disease Control and Prevention
Pan American Health Organization
World Health Organization
Regional Office for the Americas

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Table of contents

ACKNOWLEDGEMENTS	4
ABBREVIATIONS AND ACRONYMS	5
I. Background	8
II. Objectives	10
III. Methods and Data Sources	11
IV. Participation	13
V. Syphilis Tests Used by Laboratories in Americas Region	14
Direct Detection Methods	14
Serologic Testing for Syphilis	14
Point of Care (POC) Tests	16
VI. Syphilis Testing Algorithms Used by Countries	19
Syphilis Testing Algorithms Reported by Laboratories	19
National Algorithms for Syphilis Testing for Pregnant Women	20
Syphilis Testing Algorithms Used in Antenatal Care, HIV and STI Clinics	20
VII. Laboratory Service Delivery	22
Human Resources	22
Adequate Training for Laboratory Staff	22
Throughput	23
Turn-around Time	23
VIII. Quality Control and Quality Assurance for Syphilis Testing	24
Quality Control Strategies Reported by Participating Laboratories	24
IX. Syphilis Laboratory Results Reported to Surveillance Programs	27
X. Availability of Supplies and Equipment Needed for Syphilis Testing	28
Procurement	28
Equipment and Supplies Needed for Syphilis Testing	29
XI. Conclusions	32
References	35

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Abbreviations and acronyms

ANC	Antenatal Clinic
CDC	U.S. Centers for Disease Control and Prevention
CIA	Chemiluminescence Immunoassays
CSF	Cerebrospinal fluid
EIA	Enzyme Immunoassay
EMTCT	Elimination of Mother to Child Transmission
FDA	Food and Drug Administration
FTA-ABS	Fluorescent Treponemal Antibody Absorption
HIV	Human Immunodeficiency Virus
LAC	Latin America and the Caribbean
MHA-ATP	Micro-haemagglutination Assay for Treponema pallidum antibodies
MOH	Ministry of Health
PAHO	Pan American Health Organization
POC	Point of Care
PT	Proficiency Testing
QA	Quality Assurance
QC	Quality Control
RPR	Rapid Plasma Reagin
STI	Sexual Transmitted Infection
TP-HA	Treponema pallidum Haemagglutination Assay
TP-PA	Treponema pallidum Particle Agglutination Assay
USR	Unheated Serum Reagin
VDRL	Venereal Disease Research Laboratory
WHO	World Health Organization

Summary

This report presents results of the first survey assessing syphilis testing policies and practices in the Americas Region. The survey objective was to better understand laboratory practices around syphilis testing in the Region by surveying national and regional reference laboratories and a sample of large, lower-level public and private facilities. Laboratory directors or managers in the 35 member-states of the Americas Region were invited to respond to a semi-structured questionnaire, administered electronically, on questions related to syphilis tests and testing algorithms used, availability of specialized equipment and commodities needed for syphilis testing, the quality assurance and quality control (QA/QC) strategies typically employed, and challenges faced by these laboratories.

This report presents data from a total of 69 participating laboratories, representing 30 (86%) member-states. Of the 69 laboratories, 41 (59%) were national or regional reference laboratories and 28 (41%) were lower-level laboratories serving clinical facilities; three were private facilities. Regarding types of syphilis tests used, only two laboratories reported currently using direct detection methods. All laboratories reported using some type of serologic tests, and most (72%) conducted both non-treponemal and treponemal tests at their facility. Commonly used serologic assays were the rapid plasma reagin (RPR) test (reported used by 62% of laboratories), Venereal Disease Research Laboratory (VDRL) test (54% of laboratories), Fluorescent Treponemal Antibody Absorption (FTA-ABS) test (41% of laboraotries), and the Treponema pallidum Hemagglutination Assay (TP-HA) (32% of laboratories).

Most (71%) of the 69 participating facilities reported using a nationally recommended testing algorithm. However, just 76% of reference and 68% of lower-level laboratories used an algorithm that included both a non-treponemal and a treponemal test. If both tests were conducted, this was usually done using a traditional algorithm starting with a non-treponemal screening test followed by, for either reactive tests or all tests, a treponemal confirmatory test (reported by 77% of laboratories). A further 22% of laboratories reported using a reverse sequence testing algorithm (i.e., a treponemal screening test followed by a non-treponemal confirmatory test). Use of rapid, point-of-care syphilis tests was limited: Among lower-level laboratories, only 32% used any rapid syphilis tests (RSTs), with commonly reported reasons for not using RSTs including their non-inclusion in the national algorithm (26%) or in the procurement system (13%).

Almost all laboratories (94%) reported using some type of quality control (QC) strategy; and 83% of reference and 50% of lower-level laboratories participated in an external QC program. However, few laboratories conducted all recommended QC procedures. Additionally, more than half (55%) of laboratories reported at least one >30-day stock-out of an essential reagent or commodity during the previous 12 months. Laboratory directors reported a number of challenges, particularly limited opportunities for training and refresher courses for laboratory technicians.

I. Background

Syphilis, caused by the spirochaete Treponema pallidum subspecies pallidum, is an ancient disease that remains a public health concern despite the existence of inexpensive screening tests and effective antibiotic treatment regimens. Each year, 15 million new cases of syphilis occur globally, the majority in low- and middle-income countries [1]. Untreated syphilis can cause damage to the central and peripheral nervous systems, cardiovascular system, liver, bones and joints. In severe cases it results in changes in gait, neuropathy, dementia and even death [2]. Primary syphilis infection is associated with enhanced HIV acquisition and transmission. Syphilis in pregnancy is particularly devastating as, depending upon maternal stage, 50 to 80% of affected pregnancies result in a serious adverse pregnancy outcome (e.g., stillbirth, neonatal death, low birth weight infant, syphilis-infected infant) [3]. Since 2007, the World Health Organization (WHO) has promoted a global initiative aimed at elimination of congenital syphilis, but despite substantial progress an estimated 350,000 adverse pregnancy outcomes including 150,000 perinatal deaths caused by maternal syphilis occurred in 2012 [4]. Syphilis infections in adults and infants are often asymptomatic or unrecognized, but most adverse health outcomes can be prevented with early detection (through screening tests) and prompt treatment with penicillin. This is particularly important in pregnancy, during which screening and treatment is recognized as one of the most highly cost-effective public health interventions available [5].

In the Region of the Americas, WHO estimated overall syphilis prevalence in 2012 to be 0.41% for women and men, accounting for an estimated 927,000 incident cases (new infections) [1]. The prevalence of gestational syphilis in Latin America and the Caribbean (LAC) varied by country, ranging from 0.08% to 7.0% [6, 7] ,with an estimated 63,000 maternal syphilis infections during pregnancy contributing to 14,000 adverse pregnancy outcomes in the Region [3, 8]. The Americas Region has focused substantial efforts on control and elimination of congenital syphilis since the 1990s, and since 2010 has promoted dual elimination of mother-to-child transmission of HIV and syphilis using integrated programmatic approaches [6]. Through these efforts, congenital syphilis cases in the Americas have declined in many countries, and as of 2016 has been eliminated in at least one (Cuba) [9]. Nonetheless, syphilis remains a common – and entirely preventable – infection in the Region.

Currently most sexually acquired syphilis cases in LAC are concentrated among those subpopulations that are also at high risk for HIV (e.g., sex workers, migrant populations, men who have sex with men [MSM] [10]. Recently, syphilis incidence and reinfection among MSM have been rising at an alarming rate in the Region, particularly among men residing in highly urban settings; and often involving co-infection with HIV [11]. Increasing rates of syphilis in high risk groups is concerning at many levels. Detecting syphilis is critical for the individuals infected, who are often hidden or hard-to-reach owing to stigma around HIV and other sexually transmitted infections (STI). Increasing syphilis prevalence is also a public health concern due to the potential biologic synergy leading to new HIV infections and potential for further spread of syphilis into the general population. Higher general population prevalence translates into more syphilis cases among reproductive-aged women, leading to more congenital syphilis cases.

Syphilis diagnosis is based upon history, physical exam and supportive laboratory tests. The fact that most infections are asymptomatic limits use of direct detection approaches which require lesion material from very early infection. Thus, laboratory testing has relied upon serologic tests traditionally a non-treponemal screening test and treponemal confirmatory test. This testing

strategy can prove difficult in settings with limited or no access to laboratory-based services (i.e., trained technicians and specialized equipment and reagents). Attempts at improving access to syphilis diagnostics have recognized the potential efficiencies of using different algorithms based on laboratory availability and clinical situation [12]. For example, several countries in LAC have adopted an integrated approach to the prevention of mother-to-child transmission of HIV and syphilis, promoting a combined testing approach that allows for more efficient implementation of prevention services.

Several advances in syphilis testing have supported improvement in the diagnosis of syphilis. For example, although the FTA-ABS was once widely used, other treponemal tests (e.g., TPHA and TP-PA) are less dependent upon the technician's experience, and thus can be less costly, than the FTA-ABS. In addition, the recent introduction of point-of-care (POC) screening tests, often referred to as rapid syphilis tests (RSTs) has been an advantage in that these can allow prompt treatment and reduce patient loss to follow-up (i.e., lack of or late treatment) in settings such as antenatal clinic (ANC) services where early treatment is a high priority [13]. With appropriate provider training, rapid POC testing can be performed in primary care and ANC settings, allowing same day testing and treatment. Currently-available, POCs for syphilis are primarily treponemal tests which detect antibody to T. pallidum, which after infection remains positive. Thus, a positive antibody detects previous infection regardless of prior treatment, making these tests less useful in high risk populations (e.g., MSM) for whom previous infection and treatment are common. Another advance in syphilis serologic testing has been introduction of a reverse sequence algorithm using automated treponemal tests as screening assays and non-treponemal tests for confirmation [14]. Although more costly than the traditional syphilis screening algorithm on a per-test basis, reverse screening has proved efficient in settings with a large testing load and where technicians are limited (e.g., blood banks, large hospitals).

Regardless of the test types used and algorithms selected, implementing internal and external QA systems, quality improvement measures and appropriate supervision are all important to help ensure accurate testing and reduce the risk of misdiagnosis. Ideally, syphilis testing should be included as part of related clinical and public health programs (e.g., HIV, maternal and child health (MCH), reproductive health services) in order to increase program efficiencies and improve overall clinical and program services. A recent example of successful program integration has been use of dual HIV/syphilis POC tests on a single device. Another example is integration of proficiency testing programs for POC testing through use of dried tube specimens (DTS) that include both HIV and syphilis. These types of integrated models have helped several LAC countries work toward dual elimination of mother-to-child transmission of HIV and syphilis, a regional initiative that has increasingly become truly feasible [9].

II. Objectives

To date, little has been reported in the Americas Region about the state of laboratory-based syphilis testing, including the types of tests available, algorithms used, or QA strategies employed. The aim of the present report is to present the results of a regional survey that assessed syphilis testing practices among laboratories in the Pan-American Health Organization (PAHO) member countries. This survey, conducted in 2014, had objectives to understand the syphilis testing algorithms, types of diagnostic tests, and testing practices and standards currently applied by reference and large, clinical laboratories in countries within the Region of the Americas. This report is a sequel to the 2015 guidance outlining syphilis testing algorithms in different clinical settings that emphasized five key areas of focus for national programs, namely: (i) comprehensive national policies on syphilis testing and treatment, (ii) syphilis testing algorithms appropriate for specific populations and clinical or outreach settings (depending on laboratory capacity), (iii) QA of syphilis testing, (iv) ensuring availability of adequate procurement mechanisms and (v) national reporting of syphilis cases (i.e., strengthening surveillance) [12].

III. Methods and Data Sources

The 2014 survey on syphilis testing was conducted in preparation for a PAHO meeting held in April 2014 on regional laboratory needs around STI testing. Preliminary results from this survey helped inform the 2015 Guidance on Syphilis Testing in Latin America and the Caribbean [12].

For this survey, a questionnaire was developed by a collaborative technical team from the PAHO, Washington office and the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta. The intent was to understand syphilis testing practices in reference laboratories in the Region and larger, clinical laboratory settings where syphilis testing typically occurs (e.g., STI clinics, primary care clinics, HIV clinics, ANC clinics). In support of the ongoing regional initiative for the dual elimination of mother-to-child transmission of HIV and syphilis, additional questions were asked about laboratories' approaches to syphilis testing in pregnancy. The PAHO and CDC team worked with regional laboratory experts to develop a set of questions that offered insight into practices including types of syphilis tests used, testing algorithms employed, equipment and commodities available, QA in place, and challenges and needs that countries face with syphilis testing. The survey was intended to be completed by laboratory directors or managers in charge of syphilis testing at their laboratory facility, or their designees familiar with syphilis testing.

The original questionnaire was pilot-tested by three laboratory directors or managers in charge of syphilis testing in two large countries in the Region in order to assure its reliability, ability to address important and appropriate questions around syphilis testing and its ease of use. The questionnaire was developed in English and translated into Spanish for dissemination.

The final survey consisted of 94 structured, semi-structured and open-ended questions covering the following areas related to syphilis testing: type of laboratory; syphilis tests used; commodities and equipment perceived to be needed and actually available to conduct these tests; syphilis testing algorithms employed at the laboratory and (if applicable) nationally; syphilis test throughput at the facility; number of technicians available to perform syphilis testing and details on their training; procurement and distribution of commodities and equipment needed for syphilis testing, and challenges faced by laboratories, including stock outs; reporting of test results to surveillance or other public health programs; sources of funding for syphilis testing; and specific quality control and quality assurance procedures employed by the laboratories. Separate questions were asked about use of standard operating procedures, daily or weekly controls, and participation in external QA programs for syphilis testing as well as overall laboratory QA programs.

To address challenges and needs around syphilis testing, participants were asked about a structured series of common challenges, and were also provided open-ended fields to allow them to describe unique challenges or needs that were not addressed in the structured questions. Specific questions were asked about use of rapid POC tests in laboratory and clinical settings. Respondents were informed that for the purposes of this questionnaire, RPR assays were not considered to be rapid tests. Specific questions were also asked about commonly used syphilis serology tests including non-treponemal tests such as the RPR and VDRL assays, and treponemal tests such as the FTA-ABS tests, TP-HA and the Treponema pallidum Particle Agglutination (TP-PA) assay, POC assays, the enzyme immunoassay (EIA), and chemiluminescence immunoassay (CIA).

To identify survey participants, a list of eligible countries (PAHO member states) was developed, and PAHO country focal points established a contact list of all national and regional laboratories, large maternal or other public hospitals, and private hospitals in each country. For each laboratory they

were asked to identify the director or manager in charge of syphilis testing. The sampling goal of the study was to survey at least the national and large regional reference laboratory for each member country and, if possible, one or more lower-level facilities that conducted syphilis testing and one or more private laboratories from each country. There was no limit placed on the number of laboratories that could participate per country. Once the list of laboratories was identified along with potential respondents, the identified laboratory directors were contacted by email and invited to participate in the survey. Respondents were informed by e-mail that the intent was to identify the person in charge of syphilis testing at the laboratory facility, and if the email had reached another person in error to please inform the survey team and forward the survey to the correct person. The survey was administered electronically between March and August, 2014 using Survey Monkey via an online web link.

Data were analyzed using SAS version 9.3 (Cary, NC, USA) and Microsoft Excel 2013. In data cleaning, if there were areas of confusion an attempt was made to contact the respondent and reassess the response. Descriptive analyses were performed to determine proportions and percentages of responses overall and stratifying by sub-groups. Most analyses included stratification by national or large, regional (i.e., reference) laboratories and other, lower level (i.e., more clinically focused) laboratories. Additional analyses assessed responses by sub-region with countries grouped in the manner of previous PAHO reports, including North America (excluding Mexico), Central America (including Mexico), the Caribbean (including, Haiti, Guyana, Cuba and another 12 island nations), five Andean nations and five Southern Cone nations (Table 3.1). We calculated proportions, means and medians values of variables using SAS version 9.3 and reported ranges where applicable. Additional analyses assessed the use of syphilis testing by clinical setting (e.g., ANC or primary care clinics). An original intent of the survey was to compare syphilis testing practices in public and private laboratories; however, since participation by private laboratories was poor, few such analyses could be undertaken.

Table 3.1: Participating Countries by Region (Number of Countries) [Number of Laboratories]

North America (N=2) [N=2]	Central America (N=8) [N=22]	Caribbean (N=10) [N=15]	Andean (N=5) [N=16]	Southern Cone (N=5) [N=14]
Canada [1]	Belize [3]	Antigua & Barbuda [1]	Bolivia [2]	Argentina [4]
United States [1]	Costa Rica [1]	Bahamas [1]	Colombia [3]	Brazil [3]
	El Salvador [3]	Barbados [1]	Ecuador [2]	Chile [4]
	Guatemala [2]	Cuba [1]	Peru [7]	Paraguay [2]
	Honduras [3]	Dominica [1]	Venezuela [2]	Uruguay [1]
	Mexico [3]	Dominican Republic [2]		
	Nicaragua [1]	Guyana [3]		
	Panama [6]	Haiti [1]		
		Saint Lucia [2]		
		Trinidad and Tobago [2]		

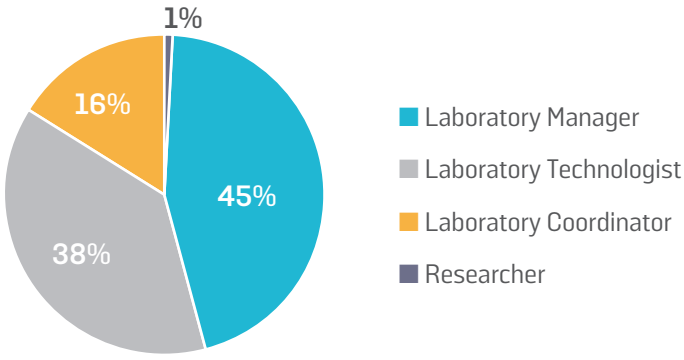
IV. Participation

A total of 69 laboratories from 30 (86%) of the 35 PAHO member states completed the survey. There were 22 (32%) laboratories from Central America, 15 (23%) from the Caribbean, 16 (23%) from the Andean nations, and 14 (20%) from South Cone nations, and two (3%) laboratories from North America. Among participating laboratories, 41 (59%) were national or regional reference laboratories and 28 (41%) were lower-level or district laboratories comprised of larger maternity hospital laboratories, private or public hospitals, or other large, primary or local health clinics (Figure 4.1).

Figure 4.1: Participating Laboratory by Level of Laboratory

Most (94%) participating laboratories were public. Just under half of the survey respondents were laboratory directors or managers (31, 45%), with other respondents either senior laboratory technologists (26, 38%) or program managers/coordinators (11, 16%) (Figure 4.2).

Figure 4.2: Job Title of Laboratory Staff Comleting the Survey



V. Syphilis Tests Used by Laboratories in Americas Region

Syphilis diagnosis is usually based upon a suggestive history and clinical findings along with supportive laboratory testing. Laboratory tests used for the diagnosis of syphilis include direct detection methods such as dark-field microscopy, direct fluorescent antibody (DFA) and nucleic acid amplification testing (NAATs), and serology tests (treponemal and non-treponemal) [2, 15].

Direct Detection Methods

Direct detection methods generally require exudates from lesions of primary syphilis, secondary syphilis or early congenital syphilis. The survey results indicated that use of direct detection methods is increasingly rare among laboratories, even in reference laboratories, in LAC.

The use of **dark-field microscopy** to demonstrate treponemes with characteristic morphology and motility in lesion exudate or tissue (e.g., placenta) is a highly specific method and a definitive diagnosis of active infection in the early stages of syphilis. However, the sensitivity can be low, ranging from about 40% to 89%, and a negative dark-field result does not exclude syphilis. Additionally, specimens may contain spiral bacteria that can be confused with T. pallidum. Dark-field examination should be performed immediately after specimen collection from moist primary chancres or secondary lesions. Lymph node aspirates and amniotic fluids can also be examined by dark-field microscopy. Although dark-field is one of the simplest and most reliable methods for the direct detection of *T. pallidum*, the technique is usually limited to certain laboratories because it requires specialized equipment and an experienced microscopist. Only two surveyed reference laboratories, one regional in Bolivia and one national laboratory in Argentina, reported using dark-field microscopy.

The **direct fluorescent antibody for *T. pallidum*** (DFA-TP) test uses a fluorescence microscope to detect spirochetes that have been stained with fluorescein-labelled anti-*T. pallidum* globulin. Specimen requirements are similar to dark-field microscopy with exudates being air dried for staining afterwards. The fluorescein-stained organisms are easier to visualize than dark-field and are not likely to be confused with other commensal spirochetes, leading to a higher sensitivity and specificity of DFA-TP compared to dark-field microscopy. However, DFA-TP also requires specialized equipment and trained personnel, and the specific fluorescein conjugate is not commercially available in most countries. Only one regional laboratory, in Canada, reported use of the DFA for diagnosis of syphilis.

Nucleic acid amplification tests (NAATs) can detect DNA from *T. pallidum* by polymerase chain reaction (PCR) from lesion exudates or infected tissue or body fluids such as cerebrospinal fluid (CSF); however, PCR is most useful for moist lesions of primary and secondary syphilis. The sensitivity of NAATs on lesion swabs is about 90%. A few PCR tests for *T. pallidum* are being used commercially, but are not yet widely available for routine clinical diagnostic use. Various PCR assays have been developed for research purposes, and one of the surveyed national laboratories in Argentina reported use of a PCR test for diagnosis of syphilis.

Serologic Testing for Syphilis

There are two general types of serologic tests used to help diagnose syphilis and to guide treatment: non-treponemal and treponemal tests. A presumptive diagnosis of syphilis generally requires a positive result from both types of tests, although clinical history and physical exam are important because serologic tests may be negative in primary syphilis or old, previously-treated syphilis infections. **Non-treponemal tests** measure immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies formed by the host in response to lipoidal material released from damaged host cells as well as to lipoprotein-like material released from the treponemes. Since

these antibodies are non-specific, false positive results can occur in some conditions such as acute febrile viral infections and some chronic autoimmune diseases. Four standard non-treponemal tests are used in current practice: the VDRL test, the RPR test, the unheated serum reagin (USR) test, and the toluidine red unheated serum test (TRUST). While non-treponemal tests tend to be highly sensitive, positive screening results should ideally be confirmed with treponemal antibody tests given their potential for false positive results. Non-treponemal tests can be false negative in early syphilis or very late syphilis. Serum is the specimen of choice for these tests, although plasma can be used in some circumstances. The VDRL test is the test of choice for use in testing cerebrospinal fluid (CSF) when neurosyphilis is suspected [16].

Non-treponemal tests may be qualitative or quantitative. Qualitative tests are performed on undiluted sera, while quantitative tests can be used as an indicator of activity of infection and can be used to monitor response to treatment. Titers are expected to decrease following effective treatment or increase in untreated, active infection. For example, a 4-fold change or higher in titer, equivalent to a change of at least two dilutions (e.g., a drop from 1:16 to 1:4) is considered an effective response to treatment assuming the same sequential tests were used (e.g., both an RPR) in the same laboratory. Most false-positive tests have low titer results (e.g., < 1:4). Therefore all reactive qualitative tests should, ideally, be titrated. Non-treponemal tests usually revert to negative within three years after effective treatment of early syphilis. However, if patients have late latent (duration of disease >2 years) or tertiary syphilis the patients' sera may continue to be seroreactive usually at low-titer, following successful treatment. This is referred to as a serofast reaction which may be lifelong.

Table 5.1: Syphilis serology tests reportedly used among laboratories in Americas Regions (N=69)

	Non-Treponemal Tests (n,%)			Treponemal Tests (n,%)					
Region	RPR	VDRL	USR	CIA	EIA	TP-PA	TP-HA	FTA-ABS	Western Blot
Overall (N=69)	43 (62)	37 (54)	2 (3)	7 (10)	17 (25)	13 (19)	22 (32)	28 (41)	1 (1)
North America (2)	2 (100)	1 (50)	0 (0)	2 (100)	1 (50)	2 (100)	0 (0)	2 (100)	0 (0)
Central America (22)	10 (45)	9 (41)	1 (5)	1 (5)	3 (14)	2 (9)	4 (18)	7 (32)	0 (0)
Caribbean (15)	13 (87)	6 (40)	0 (0)	1 (7)	6 (40)	4 (27)	4 (27)	1 (7)	0 (0)
Andean (16)	13 (81)	7 (44)	0 (0)	2 (13)	3 (19)	4 (25)	6 (38)	7 (44)	0 (0)
Southern Cone (14)	5 (36)	14 (100)	1 (7)	1 (7)	4 (29)	1 (7)	8 (57)	11 (79)	1 (7)

RPR: Rapid Plasma Reagin, VDRL: Venereal Disease Research Laboratory, USR: Unheated Serum Reagin, CIA: Chemiluminescence Immunoassays , EIA: Enzyme Immunoassay, TP-PA: Treponema pallidum Particle Agglutination Assay, FTA-ABS: Fluorescent Treponemal Antibody Absorption

The most commonly reported non-treponemal test used among the surveyed laboratories was the RPR (43 laboratories or 62%), followed by the VDRL (37 laboratories or 54%) and the USR (2 laboratories or 3%). No laboratory reported using the TRUST (Table 5.1). The survey did not include specific questions on use of qualitative and quantitative non-treponemal testing. It found that 65 (94%) of the 69 participating laboratories conducted at least one non-treponemal test, including 38 (93%) of the 41 national or regional laboratories and 27 (96%) of the 28 lower level laboratories.

Treponemal antibody tests use whole *T. pallidum* organisms or specific components as an antigen. In the past, these have been used as a means of confirming reactive results of non-treponemal tests (traditional algorithm). More recently, reverse sequence algorithms have been employed that involve screening with treponemal tests and confirming reactive results with non-treponemal tests. This approach is particularly common in large volume laboratories using automated EIAs or CIAs, or in small clinics using rapid, POC treponemal tests.

Other commonly used serologic treponemal tests include the TP-PA, the TP-HA, the FTA-ABS assay, and the microhemagglutination assay for *T. pallidum* antibodies (MHA-TP). Additionally, the IgG western blot using a lysate of *T. pallidum* whole cells is a complex but highly sensitive and specific test that has been used as a confirmatory, treponemal test. As is the case for all serologic tests, laboratory-based treponemal tests require trained laboratory personnel and at least basic laboratory capacity, including specific reagents and equipment for each test, refrigerators, centrifuge and specialized equipment such as shakers, special slides or other tools.

Treponemal tests are highly specific, although no serological test can differentiate between the *T. pallidum* subspecies causing venereal syphilis and the other non-venereal trepanematoses (e.g., yaws, bejel and pinta). As mentioned, treponemal antibody persists for life, even after effective treatment, and thus treponemal tests cannot distinguish between recent, active infection and previously treated infections. Thus, ideally treponemal testing should be paired with a non-treponemal test to distinguish between active and past infection. Serum is the specimen of choice for treponemal tests. For testing for presence of neurosyphilis using cerebrospinal fluid (CSF, both the VDRL (prepared) and the FTA-ABS have been used to exclude neurosyphilis [17] although VDRL is considered the test of choice.

The most commonly reported treponemal test used by surveyed laboratories was the FTA-ABS reported by 28 (41%) laboratories, and 22 (32%) laboratories reported using the TPHA (Table 5.1). Of note, the FTA-ABS is amore technical demanding and subjective test, and in inexperienced hands can be less sensitive, than the TP-HA or TP-PA; it is also more costly [18]. The survey results found that 56 (81%) of the 69 participating laboratories conducted at least one treponemal test, including 35 (85%) of the 41 national or regional laboratories and 21 (75%) of the 28 lower level laboratories.

Among national/regional reference laboratories, 11 of 41 laboratories (27%) only used a single type of serologic test (i.e., did not use a confirmatory test): 4 reference laboratories reported conducting no non-treponemal testing, and 7 lower-level laboratories reported conducting no treponemal testing (Table 5.2).

Point of Care (POC) Tests

Rapid syphilis tests (RSTs) employ finger-prick, whole blood samples which allow testing to be done at the clinic. These are therefore often referred to as "point-of-care" (POC) tests. Most RSTs that are currently marketed are treponemal tests, although at least one treponemal/non-treponemal test is available in some countries (Chembio Dual Path Platform Syphilis Screen and Confirm). Table 5.3 shows use of RSTs in the participating laboratories. Of the 69 laboratories, 28 (41%) reported using at least one RST, representing 16 (53%) of the 30 reporting countries. Of note, most (68%) facilities using POC syphilis tests were national or regional laboratories (i.e., not laboratories associated with a clinic) suggesting that the RSTs may be used in place of

laboratory-based treponemal tests as confirmatory tests. RSTs are more costly and less sensitive than TP-PA or TP-HA, and were primarily developed for use in clinical settings in which screening tests are required and timeliness of results is important (e.g., ANC) [19, 20]. The survey indicated that only 9 (32%) facilities were lower-level laboratories, which are the facilities most likely to be linked to clinical settings, used RSTs (Table 5.3). Ideally, in the future more lower level laboratories will have access to high quality RSTs to support immediate results. This is particularly relevant for ensuring early syphilis testing for all pregnant women in ANC settings, in order to facilitate prompt treatment for those testing positive, reducing likelihood of an adverse fetal outcome caused by syphilis exposure. On the other hand, use of RSTs in national and regional reference laboratories is somewhat concerning if these replace more accurate and less expensive laboratory-based treponemal tests such as the TP-PA or TP-HA.

Among the 41 institutions that did not perform POC syphilis tests, reasons given for not using the RSTs are shown in Figure 5.1. Approximately one-fourth (26%) of the respondents indicated that the main reason for not using RSTs was that the tests were not included in the national algorithm for syphilis testing. Other commonly reported reasons for not

Table 5.2: Types of Syphilis Test Conducted by Laboratories, by Level

Type of Test	Type of laboratory		
	All (N = 69)	National/Regional (n = 41)	Lower level (n= 28)
	(n, %)	(n, %)	(n, %)
Non- Treponemal Test			
RPR	43 (62)	25 (61)	18 (64)
VDRL	37 (54)	19 (46)	18 (64)
USR	2 (3)	1 (2)	1 (4)
Treponemal Test			
FTA	28 (41)	20 (49)	8 (28)
TPPA	13 (19)	12 (29)	1 (4)
TPHA	21 (30)	15 (37)	6 (21)
EIA	17 (25)	12 (29)	5 (18)
CIA	7 (10)	3 (7)	4 (14)
Rapid Treponenal test	28 (41)	19 (46)	9 (32)
Only Non-Treponemal Test(s)	13 (19)	7 (17)	6 (21)
Only Treponemal Test(s)	5 (7)	4 (10)	1 (4)

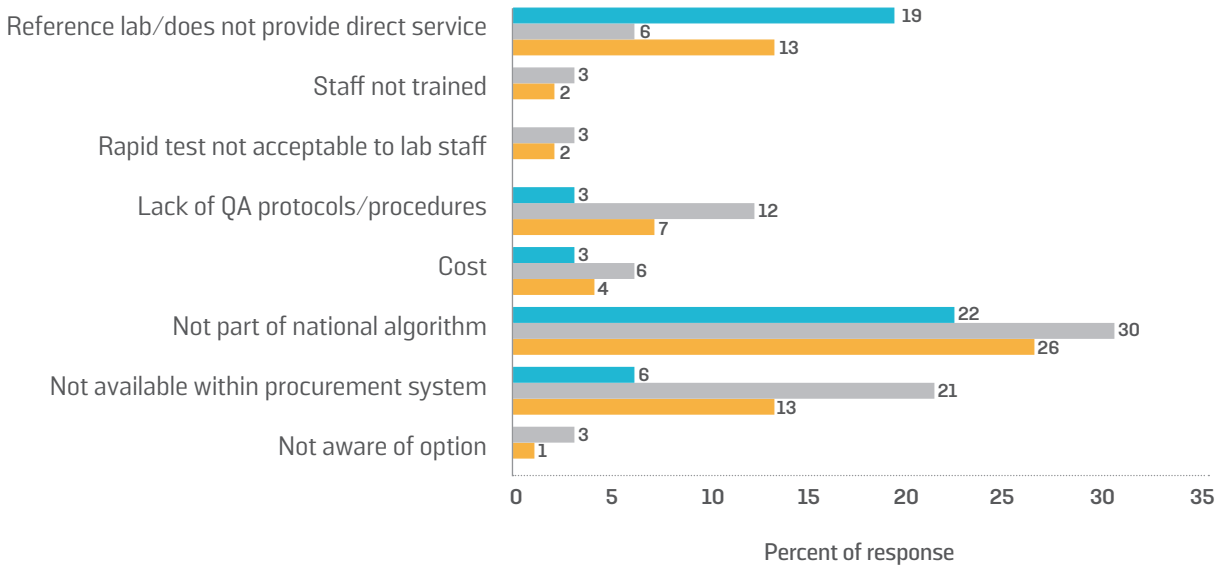
RPR: Rapid Plasma Reagin, VDRL: Venereal Disease Research, Laboratory, USR: Unheated Serum Reagin, FTA-ABS: Fluorescent Treponemal Antibody Absorption, TPPA: Treponema pallidum Particle Agglutination Assay, TPHA: Treponema pallidum Haemagglutination Assay, EIA: Enzyme Immunoassay, CIA: Chemiluminescence Immunoassays

Table 5.3: Use of Rapid Treponemal Testing among Participating Laboratories by Level (N = 69)

Type of laboratory	N	%
All (69)	28	41
National/Regional level	19	68
Lower level	9	32

using RSTs were that the tests were not included in the procurement system (13%), and that the laboratories did not provide a direct service to patients (13%). Few laboratories cited cost or lack of staff training as reasons for not using RSTs.

Figure 5.1: Reasons for not using Rapid Syphilis Tests Reported by 41 Laboratories, Regional Survey of Syphilis Testing in the Americas Region, 2014



When participants were asked about acceptable settings for RSTs implementation, the majority (59%) of respondents reported that mobile outreach programs for at-risk populations was an acceptable setting, as were STI clinics (51%), HIV clinics (49%), ANC settings (46%), primary health care clinics (46%), and emergency wards (41%), respectively. Additionally, 37% of respondents reported that they believed laboratories were an acceptable setting for use of RSTs.

Of the 28 laboratories that used rapid syphilis tests, only four reported using them in a health care setting to promote prompt treatment and reduce loss of follow-up [19, 20]. Of note, five reference laboratories used rapid tests to confirm reactive non-treponemal tests. As discussed earlier, rapid syphilis tests may be especially useful in ANC settings where pregnant women with positive test results can receive treatment at the same visit for prevention of mother-to-child transmission of syphilis.

VI. Syphilis Testing Algorithms Used by Countries

The 2015 syphilis testing survey asked laboratories about any standardized syphilis testing algorithm employed in their laboratory and any nationally recommended algorithm used among antenatal women.

Syphilis Testing Algorithms Reported by Laboratories

Testing algorithms reported by the 69 participating laboratories are shown in table 6.1. While most laboratories reported use of an optimal strategy that employed both a screening and confirmatory testing for syphilis, 13 laboratories (10 reference, 3 lower-level) did not. The most commonly used testing algorithm, reported by 33 (48%) laboratories, was a traditional algorithm using a non-treponemal screening test with reactive tests confirmed by a laboratory-based treponemal test. An additional 11 (16%) laboratories used non-treponemal screening tests with all tests confirmed by a laboratory-based treponemal test, and a further nine (13%) facilities employed a non-treponemal tests with reactive tests confirmed by a rapid treponemal test. Fifteen (22%) laboratories used a type of reverse screening algorithm employing a treponemal test first followed by a non-treponemal test. Seven laboratories used laboratory based serologic tests, while eight used a rapid treponemal test confirmed with a non-treponemal test. Thirteen (19%) laboratories used a sub-optimal algorithm that included only one type of serologic, limiting the ability to accurately diagnosis syphilis infection or to determine whether it was recent or old (e.g., previously treated) infection. This approach is particularly limiting in diagnosing syphilis in sub-populations likely to have had previous infection (e.g., STI clinic patients, HIV patients, MSM, female sex workers). Of note, some laboratories used more than one syphilis testing algorithm (i.e., in different clinical settings)

Table 6.1: Syphilis Testing Algorithms Reported by Participating Laboratories (N=69)*

	N	%
RPR or VDRL with REACTIVE tests confirmed by a lab-based treponemal testing (e.g., TPHA, TP-PA, FTA-ABS, EIA, CIA)	33	48
RPR or VDRL with ALL tests confirmed by a lab-based treponemal test (e.g., TPHA, TPPA, FTA-ABS, EIA, CIA)	11	16
RPR or VDRL with reactive tests confirmed by a rapid treponemal test	9	13
Lab-based treponemal test (TP-PA, FTA-ABS, EIA, CIA) with reactive tests confirmed by an RPR or VDRL	6	9
Lab-based treponemal test with all tests confirmed by RPR	1	1
Rapid treponemal test with reactive tests confirmed by RPR or VDRL	8	12
RPR or VDRL only	10	14
Lab-based treponemal test only (e.g. TPHA, TP-PA, FTA-ABS, EIA, CIA)	1	1
Rapid treponemal test only	2	3

* Multiple choices possible, thus columns do not add to 100%

National Algorithms for Syphilis Testing for Pregnant Women

Forty nine (71%) participating laboratories reported the existence of a recommended national algorithm for syphilis testing in pregnant women. The most commonly used algorithm during pregnancy was a traditional approach, reported by 40 (82%) of the 49 laboratories. An additional 7 (17%) laboratories used a reverse screening algorithm. Two laboratories reported using more than one algorithm in ANC settings.

Syphilis Testing Algorithms Used in Antenatal Care, HIV and STI Clinics

Table 6.2 shows differences in syphilis testing algorithms serving ANC programs, HIV programs, and STI programs by laboratories supporting these services. Since patients seeking HIV or STI clinical care are often higher risk and may have been exposed (and treated) for syphilis in the past, an algorithm including both a non-treponemal test and confirmatory treponemal test is the recommended approach [2, 12]. In the survey, for HIV and STI programs, the most commonly reported algorithm was the use of a non-treponemal test alone (RPR or VDRL), followed by use of a rapid treponemal test only, or a non-treponemal test with reactive tests confirmed by a laboratory-based treponemal test. As noted earlier, clinical laboratories serving these populations that currently use a non-treponemal test alone could consider adopting an algorithm that includes a rapid treponemal test as confirmation of the current non-treponemal tests. Clinics serving high risk populations that use only a rapid treponemal test may consider changing their algorithm for one that can better identify recent, active infections.

Table 6.2: Difference in Syphilis Testing Algorithms Reported by All Laboratories Serving Antenatal Clinics (ANC), HIV Programs (HIV) and Sexually Transmitted Infection(STI) Clinics

Type of Clinic:	ANC (n= 54)	HIV (n=47)	STI (n=53)
Reported Syphilis Testing Algorithms:	**n (%)**	**n (%)**	**n (%)**
RPR or VDRL with REACTIVE tests confirmed by a lab-based treponemal testing (e.g., TP-HA, TP-PA, FTA-ABS, EIA, CIA)	28 (52)	5 (11)	5 (9)
RPR or VDRL with ALL tests confirmed by a lab-based treponemal test (e.g., TP-HA, TP-PA, FTA-ABS, EIA, CIA)	5 (9)	1 (2)	2 (4)
RPR or VDRL with reactive tests confirmed by a rapid treponemal test	1 (2)	1 (2)	4 (8)
Lab-based treponemal test (e.g., TP-HA, TP-PA, FTA-ABS, EIA, CIA) with reactive tests confirmed by an RPR or VDRL	4 (7)	1 (2)	0 (0)
Rapid treponemal test with reactive tests confirmed by RPR or VDRL	1 (2)	0 (0)	0 (0)
RPR or VDRL only	2 (4)	26 (55)	26 (49)
Lab-based treponemal test only (e.g., TPHA, TP-PA, FTA-ABS, EIA, CIA)	0 (0)	2 (4)	2 (4)
Rapid treponemal test only	5 (9)	6 (13)	8 (15)
Other	4 (7)	2 (4)	1 (2)
No algorithm or don't know	4 (7)	3 (6)	5 (9)

RPR: Rapid Plasma Reagin, VDRL: Venereal Disease Research Laboratory, TPHA: Treponema pallidum Haemagglutination Assay, TP-PA: Treponema pallidum Particle Agglutination Assay, FTA-ABS: Fluorescent Treponemal Antibody Absorption, EIA: Enzyme Immunoassay, CIA: Chemiluminescence Immunoassays

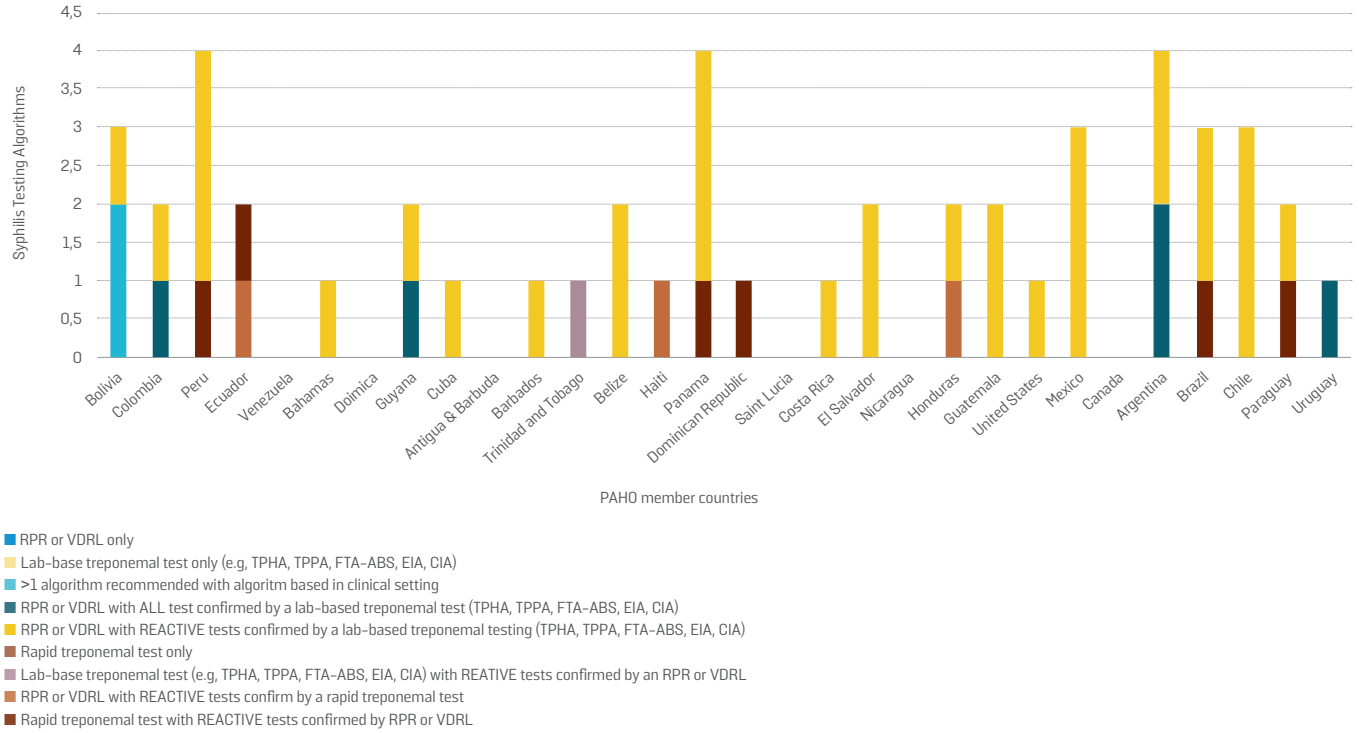
In laboratories serving ANC populations, the most commonly used algorithm was a non-treponemal test with reactive tests being confirmed by a laboratory-based treponemal test. The survey indicated that very few laboratories or clinics had yet adopted rapid testing strategies. Several laboratories serving ANC clinics (7%), HIV programs (6%) and STI clinics (9%) reported that they did not know or were unsure of an existence of a national algorithm for syphilis testing for pregnant women.

In contrast to clinics serving populations at higher risk for syphilis, ANC settings serve generally low risk women where the need to detect and treat syphilis promptly (ideally in the clinic setting) is imperative to protect the fetus. Use of RSTs alone to identify women for treatment could be justified in these settings because the risk of a syphilis infection in pregnancy is very high (>50% of infections result in a severely adverse pregnancy complication) while the risk of treatment with penicillin is very low [4, 21]. Use of RSTs can help support countries to reach the target of at least 95% syphilis testing of all pregnant women in order to eliminate mother-to-child transmission of syphilis. Use of a combination test, such as the dual rapid syphilis/HIV tests on a single device, could help support achieving the 95% testing coverage targets for both HIV and syphilis among pregnant women [22].

Among the 54 laboratories that supported ANC services (often in addition to other clinical services), 77% reported using a traditional algorithm of a non-treponemal test with either all tests or reactive tests confirmed with laboratory-based treponemal testing. Laboratories were also asked about existence of a "national algorithm for syphilis testing in pregnant women." Among the 67 laboratories from 30 countries reporting on this question, 24 (80%) countries with 49 (71%) laboratories had a national algorithm for syphilis testing for pregnant women. Details on reported national algorithms for countries are shown in Figure 6.1.

Of note, among the 24 countries reporting the existence of a national algorithm for syphilis testing for pregnant women, 14 (58%) used one algorithm and 10 (42%) countries used more than one algorithm.

Figure 6.1: National Algorithm on Syphilis Testing for Pregnant Women (N=24 countries, n=49 laboratories)



VII. Laboratory Service Delivery

Ensuring delivery of quality laboratory services requires sufficient laboratory staff who are adequately trained in the standard procedures for specific syphilis tests as well as in basic laboratory systems including QC and QA, biosafety and adequate forecasting for procurement of commodities. New service staff should also be trained in the routine maintenance of the various equipment required for specific tests.

Human Resources

Having a sufficient number of appropriately trained personnel is a fundamental element in delivery of quality laboratory services. The laboratories participating in the survey reported an overall median of 5 (range, 0 – 30) laboratory and clinical staff involved in performing syphilis testing in their facility (Table 7.1). The number of trained personnel conducting syphilis testing was slightly higher in district and lower level laboratories (median, 5.0) than national/regional laboratories (median, 4.0). Among responding national/regional laboratories, fewer staff were available from countries of the Andean sub-region (median, 2.0) and more were available in North America (median, 7.5).

Adequate Training for Laboratory Staff

National/regional laboratory participants reported that on average most staff performing syphilis testing had received specialized training in that area (Table 7.1). For district and other lower-level laboratories, fewer staff had been trained: on average 25% in the countries of the Southern Cone, 82% in Andean countries; 83% in Caribbean countries/territories; and 100% in Central American countries (no district/lower level laboratories from North America participated in the survey). While few survey participants (5 laboratories or 6%) reported needing additional staff to perform syphilis testing, many (28 laboratories or 36%) reported that training of laboratory personnel was among their most pressing needs. Commonly reported requests were technical

Table 7.1: Laboratory Personnel Performing Syphilis Testing (median, range) and who had Received Training in Syphilis Testing (median, range)

Survey Participants	Staff Performing Syphilis Testing (Median, Range)	Staff Trained in Syphilis Testing (Median, Range)
Overall (N=68)*	**5.0 (0 – 30)**	**5.7 (0 – 20)**
National/Regional lab (N=41)	**4.0 (1 – 19)**	**4.5 (0 – 19)**
North (2)	7.5 (5 – 10)	7.0 (5 – 10)
Central (22)	5.0 (3 – 19)	7.0 (4 – 19)
Caribbean (15)	5.0 (3 – 12)	5.0 (3 – 12)
Andean (15)	2.0 (1 – 4)	2.5 (0 – 4)
Southern Cone (14)	4.0 (2 – 6)	4.0 (2 – 6)
District/Lower Lab (N=27)	**5.0 (0 – 30)**	**5.0 (0 – 20)**
North (2)**		
Central (22)	3.5 (0– 7)	3.5 (2– 7)
Caribbean (15)	6.0 (5 – 20)	5.0 (0– 20)
Andean (15)	8.5 (3 – 20)	7.0 (3– 15)
Southern Cone (14)	4.0 (2 – 30)	1.0 (0– 9)

(*) 1 laboratory did not respond to the question / (**) There was no participating laboratory from district/local level

assistance in training around conducting RPR tests and FTA-ABS tests, as well as training in QC procedures and continuous quality improvement.

Throughput

Participating laboratories were located in large and small countries, and had different responsibilities (national/regional reference laboratories and district/lower levels clinical facilities), and thus the range of specimens processed was great. Therefore, we report on median number of samples and range to get a sense of the distribution of services. Of the 67 laboratories that answered the survey question, they reported testing a median of 300 samples (range 1 – 41,000) per month[1]. Participating national and regional reference laboratories reported a median of 215 samples per month; range 1 – 41,000. In general, district/lower level laboratories (primarily clinical facilities) reported more testing, a median of 400 samples per month; range 1 – 37,000) per month[2]. Most laboratories (60%) conducted syphilis testing at least daily or "on demand." Another 13% conducted testing at least twice a week and typically more often; and an additional 13% reported conducting syphilis testing weekly. Six of the participating laboratories reported conducting syphilis testing less than weekly, including three that tested every two weeks and three that tested monthly. Of these six laboratories, five were in Central America or the Caribbean.

Turn-around Time

Laboratories were asked about how the time required to provide test results once samples were run. Of the 69 participating laboratories, 48% reported they were able to provide syphilis testing results back to clinics within a day, whereas 4% of surveyed laboratories required more than seven days. Laboratories that served STI clinics at district or lower levels reported a median 5-hour turnaround time for test results compared to a median 24-hour turnaround time for laboratories at national or regional reference laboratories. The Central American sub-region laboratories reported the shortest turnaround times for test results with a median of only 3 hours. However, for the six laboratories conducting syphilis tests every two weeks or monthly, final results were not available to clinics for 5 weeks or more. This was time to return of test results to the facility, and may not have reflected the full time elapsed before results were provided to patients.

Thirty (45%) laboratories reported offering same-visit testing and treatment. Lower- level laboratories serving STI and ANC patients tended to report shorter turnaround times (median 5 hours for STI clinics, 4 hours for ANC clinics). Of the 54 laboratories that served ANC clinics, 28 (52%) reported that their laboratories had the capacity to provide same day testing and treatment. Same visit testing and treatment is an important precaution strategy as pregnant women with positive tests may be lost to follow-up and thus not receive sufficiently timely treatment to prevent congenital syphilis. Although this 4-5 hours is a short time for turnaround of results, it is more likely that most STI and ANC patients do not remain at the clinic for this long time.

[1]41,000 processed specimens was reported by a large, regional laboratory in Canada

[2]37,000 processed specimens was reported by a large district laboratory

VIII. Quality Control and Quality Assurance for Syphilis Testing

Ensuring the quality of the tests used and the testing done are critical aspects of all laboratory testing program, including syphilis testing. *Quality control* (QC) refers to the procedures used for each laboratory assay to assure that each test run is valid and results are reliable. Examples of QC procedures include developing policies at the national level to ensure basic standardized public health laboratory procedures and operations occur in areas of management structure, biosafety, forecasting commodities and equipment needs, procurement of tests and reagents, maintenance of equipment, specimen collection and processing, laboratory testing and reporting and documentation of results.

For syphilis testing, national policies can ensure that the tests used are of sufficiently high quality to provide accurate results. This may include use of only tests that have achieved a certain international standard (e.g., European Union CE-mark, U.S. Food and Drug Administration (FDA) approval, WHO prequalification), or at least have been evaluated on performance characteristics (sensitivity/specificity) against a known standard and by a reputable laboratory.

Internal QC for syphilis testing refers to standards ensuring the test is correctly conducted according to manufacturers' recommendations. QC includes written standard operating procedures for each test describing how equipment is maintained and tests are performed in the laboratory setting, and use of control samples. *External quality assurance* (EQA) refers to systems in place to ensure the level of testing by laboratory technicians or clinical providers is accurate and proficient (sometimes referred to as "proficiency testing"). At a country level, EQA is a system provided by a high level (e.g., national) laboratory to ensure that lower level testers are achieving appropriate results. This system may be done through use of dried tube specimens (DTS) sent to laboratory or clinical staff conducting tests, or through direct observation or other means. EQA can also refer to the program national laboratories belong to in order to ensure the national laboratory testing achieves international standards [23-25].

Quality Control Strategies Reported by Participating Laboratories

Overall, 65 (94%) of laboratories, including 97% of national/regional laboratories and 89% of lower level laboratories, reported using one or more standard QA/QC procedures for syphilis. The laboratories supporting antenatal care (ANC) services, STI and HIV clinics reported similar participation rates on using standard syphilis QA/QC procedures (83% for ANC clinics, 83% for STI clinics and 81% for HIV clinics).

Regarding internal QC, the surveyed reference laboratories at the national or regional level reported written standard operating procedures (SOPs) were available on site more commonly than lower level facilities (76% compared to 50%, respectively). Most laboratories (83%) performed daily serologic testing using controls. National and regional reference laboratories and district or lower laboratories reported similar performance of daily syphilis serologic testing using controls (85% and 79%, respectively). Daily serologic testing using controls were reported more often from laboratories in Central America (23%) than other regions (South Cone 21%, the Caribbean 13% and Andean 13%).

Seventy percent of responding laboratories reported participation in an external QC program; including 83% of the national or regional laboratories (reference laboratories) and 50% of the district or lower-level laboratories (primarily clinical laboratories). Half of participating laboratories reported conducting routine on-site observations of laboratory testing

performed in their facilities. More than half (59%) of the national and regional laboratories reported using syphilis proficiency testing panels compared to 18% of the district or lower laboratories (Table 8.1). However, it was not entirely clear whether this response referred to panels that were made by the national or regional laboratories in support of the lower-level laboratories, or to the participation by the national or regional laboratories in an external (e.g., international) proficiency testing program.

Table 8.1 Commonly used QA/QC Strategies Reported by Participating Laboratories (n = 69)

	Overall (n= 69)	National/ Regional (n = 41)	District/ Lower * (n= 28)
	n (%)	n (%)	n (%)
Written standard operating procedures on-site	45 (65)	31 (76)	14 (50)
Daily testing using controls	57 (83)	35 (85)	22 (79)
Routine, periodic observation of staff performing testing	35 (51)	22 (54)	13 (46)
Routine checks/maintenance of equipment	44 (64)	29 (71)	15 (54)
Routine procurement of reagents and/or test kits	37 (54)	22 (52)	15 (56)
Use of proficiency testing panels	29 (42)	24 (59)	5 (18)
Dried tube specimens to assess rapid treponemal tests	2 (3)	1 (2)	1 (4)
Participate in external quality control program	48 (70)	34 (83)	14 (50)
No QA/QC strategies used at facility	1 (1)	1 (2)	0 (0)

(*) District/Lower laboratories include district laboratory, health care clinic's laboratory, hospital and university laboratories

Surveyed laboratories reported a median number of five laboratory and clinical staff (range, 0 – 30) who performed syphilis testing (any type) at their facility. Seventy three percent of the 69 participating laboratories reported their staff was trained on laboratory-based non-treponemal tests, 49% on laboratory-based treponemal tests, and 46% on rapid syphilis tests. The proportion of staff trained at the national or regional reference laboratories was roughly similar to the proportion trained at district and lower level facilities (Table 8.2). Gaps in training on critical aspects of syphilis testing were reported in all sub-regions.

Table 8.2: Proportion of Clinics with Access to Different Types of Training, by Sub-region (n= 69)

	Overall (n= 69)	North (n= 2)	Central (n=22)	Caribbean (n= 15)	Andean (n=16)	Southen Cone (n= 14)	National/ Regional (n=41)	District/ Lower (n=27)*
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Laboratory-based non-treponemal tests (e.g. RPR, VDRL)	51 (73)	1 (50)	17 (77)	14 (93)	10 (63)	9 (64)	30 (73)	21 (78)
Laboratory-based treponemal tests (TP-PA, TP-HA, FTA-ABS, EIA)	34 (49)	1 (50)	11 (50)	8 (53)	7 (44)	7 (50)	22 (54)	12 (44)
Rapid treponemal tests	34 (49)	0 (0)	11 (55)	9 (60)	7 (44)	7 (50)	22 (54)	12 (44)
Standard operating procedures for QA and QC, including maintenance of rotator and shaker	32 (46)	1 (50)	11 (50)	10 (67)	5 (31)	5 (36)	22 (54)	9 (33)
models/materials of any type	4 (6)	1 (50)	0 (0)	0 (0)	3 (19)	0 (0)	4 (10)	0 (0)

(*) There was one missing

RPR: Rapid Plasma Reagin, VDRL: Venereal Disease Research Laboratory, TP-HA: Treponema pallidum Haemagglutination Assay, TP-PA: Treponema pallidum Particle Agglutination Assay, FTA-ABS: Fluorescent Treponemal Antibody Absorption, EIA: Enzyme Immunoassay, QA: Quality Assurance, QC: Quality Control

Overall, 46 laboratories (67%) reported linking syphilis QA/QC strategies to HIV testing. These included 24 (34%) laboratories that linked syphilis testing with HIV testing in staff training programs.

IX. Syphilis Laboratory Results Reported to Surveillance Programs

Public health surveillance is the ongoing, systematic collection, analysis, interpretation, and dissemination of data for public health action. Public health surveillance supports program planning and evaluation [26, 27]. For example, for the Americas regional initiative on elimination of mother-to-child transmission of HIV and syphilis (EMTCT), surveillance data on syphilis testing and treatment among pregnant women is important to monitor progress toward elimination [28]. Similarly, data on primary and secondary syphilis in the general population or key populations can help countries understand the burden of new syphilis infections.

Among the laboratories participating in the syphilis testing survey, 88% of national or regional laboratories and 89% of district or lower level laboratories responded that they reported syphilis results to a public health surveillance system. The participating laboratories were most likely to report syphilis results to the STI program (49% for national/regional and 39% for district/lower laboratories), followed by the communicable disease program, HIV program, or another surveillance program. District and lower laboratories reported to the maternal and child health (MCH) program more commonly than did the national or regional laboratories (14% compared to 7%, respectively). However, reporting to these programs varied considerably by sub-region (Table 9.1).

Table 9.1: Syphilis Test Results that are Reported to Various Surveillance Programs by Laboratory Types (n=69)*

	Overall	North	Central	Caribbean	Andean	South Cone
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
National/regional laboratories	n = 41	n = 2	n = 14	n = 10	n = 9	n = 6
Any program	36 (88)	1 (50)	11 (79)	9 (90)	9 (100)	6 (100)
STI program	20 (49)	0 (0)	6 (43)	5 (50)	6 (67)	3 (50)
MCH program	3 (7)	0 (0)	2 (14)	1 (10)	0 (0)	0 (0)
HIV program	8 (20)	0 (0)	3 (21)	3 (30)	2 (22)	0 (0)
Communicable Disease program	8 (20)	1 (50)	3 (14)	2 (20)	0 (0)	2 (17)
National surveillance program	8 (20)	0 (0)	4 (29)	1 (10)	3 (33)	0 (0)
No report to any where	5 (12)	1 (50)	3 (21)	1 (10)	0 (0)	0 (0)
District/lower laboratories	n = 28	n = 0	n = 8	n = 5	n = 7	n = 8
Any program	25 (89)	NA	7 (88)	5 (100)	6 (86)	7 (88)
STI program	11 (39)	NA	1 (13)	1 (20)	4 (57)	5 (63)
MCH program	4 (14)	NA	2 (25)	0 (0)	1 (14)	1 (13)
HIV program	3 (11)	NA	1 (13)	0 (0)	2 (29)	0 (0)
Communicable Disease program	5 (18)	NA	1 (13)	2 (40)	0 (0)	2 (25)
National surveillance program	4 (14)	NA	2 (25)	2 (40)	0 (0)	0 (0)
No report to any where	3 (11)	NA	1 (13)	0 (20)	1 (14)	1 (13)

NA- not applicable

* Multiple choices possible, thus columns do not add to 100%

X. Availability of Supplies and Equipment Needed for Syphilis Testing

Procurement

Processes for procurement of diagnostics and related laboratory items consist of three phases. The first phase is planning including forecasting needs, procurement planning, product selection, product quantification, and budgeting. The second phase is implementation with product specification and vendor selection. The last phase is monitoring and evaluation of supplier performance [29].

The survey collected data on procurement mechanisms for syphilis test kits and reagents; and funding sources that exist for syphilis testing in the participating countries.

Regarding common systems for test kit and supply procurement for syphilis testing, 48% of the participating laboratories reported that the national central distributor through the health system procured the test kits and supplies then delivered these to the laboratories based upon their requests. Twenty-eight percent of the respondents reported that syphilis test kits were purchased directly from in-country private companies and 17% reported that they were procured by the STI program or the integrated STI/HIV program. National level laboratories were more likely to receive test kits and supplies from the STI program (22% of the national laboratories vs. 11% of the district/lower laboratories) (Table 10.1).

Table 10.1: Common System for Test Kits and Supplies Procurement (N = 69)

	Overall (n=69)	National level (n = 41)	District/Lower level (n=28)
	(n=28)	n (%)	n (%)
From a national central distributor through the health system, based on institution request	33 (48)	21 (51)	12 (43)
From a national central distributor through the health system on a routine basis (without request)	2 (3)	1 (2)	1 (4)
From the HIV program, separately from other health commodities and supplies	7 (10)	3 (7)	4 (14)
From the STI program or integrated STI/HIV program	12 (17)	9 (22)	3 (11)
From the Maternal Child Health program	0 (0)	0 (0)	0 (0)
From the Reproductive Health program	0 (0)	0 (0)	0 (0)
From the non-governmental agencies or donors	4 (6)	4 (10)	0 (0)
Directly purchased from private companies within our country	19 (28)	12 (29)	7 (25)
Directly purchased from international companies	7 (10)	3 (7)	4 (14)

Equipment and Supplies Needed for Syphilis Testing

Thirty nine percent of 43 laboratories performing VDRL testing did not have the orbital rotator required for these tests. Some laboratories reported using a lateral rotator that is not recommended [30, 31] (Table 10.2). Only 23 (53%) of the 43 laboratories doing VDRL could correctly access the rotator speed that should be used (180 RPM ±2 RPM for 4 minutes under a humidifying cover is recommended), ideally checked daily or at the very least weekly. Current guidelines recommend daily calibration of orbital rotators. Furthermore, of the 17 laboratories reporting rotator age, a third were more than 15 years old. Only 29 laboratories reported having microscopes used for direct detection of treponemes, some of them more than 25 years old.

Table 10.2: Equipment and Supplies for VDRL Serologic Tests

	Overall (n=43)	National (n=22)	Regional (n=21)	North America (n=1)	Central (n=12)	Caribbean (n=8)	Andean (n=10)	South Cone (n=12)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Orbital (circular) rotator	26 (60)	12 (55)	14 (67)	0 (0)	5 (42)	6 (75)	5 (50)	10 (83)
Lateral (back and forth) rotator	8 (19)	6 (27)	2 (10)	1 (100)	3(25)	2 (25)	1 (10)	1 (8)
Kline slides	13 (30)	5 (23)	8 (38)	0 (0)	5 (42)	2 (25)	3 (30)	3 (25)
VDRL slides	20 (47)	12 (55)	8 (38)	0 (0)	4 (33)	3 (38)	6 (60)	7 (58)
Home-make slides	2 (5)	2 (9)	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)	1 (8)

Development of a procurement plan can help eliminate stock-outs of critical reagents and supplies needed for laboratory testing. Identification of the quantity of supplies needed should be based on multiple sources such as types of test procedure conducted, historical consumption data, epidemiological information and input from laboratory staff and should take into account local changes (e.g., increased testing in ANC clinics if programs promote elimination of congenital syphilis, a change in guidelines, or a local epidemic such as Zika). Forecasting refers to projections of quantities of product required to meet demand for future time period. Forecasts are most often made for a 1 to 2 year period. Demand data from the field are critical for forecasts and poor quality data may lead to inaccuracies, resulting in stock-outs or oversupply and waste of resources that may expire.

In the survey, stock-outs were defined as an event that causes inventory to be exhausted. Stock-outs of test kits and supplies were reported by 69 participating laboratories. Overall, during the 12 months preceding the survey, stock-outs were reported by 55% of laboratories performing the VDRL testing, 46% of laboratories performing EIA, and 30% of the laboratories conducting the RPR test. Additionally, 26% of laboratories conducting RPR tests reported stock-outs of RPR cards. Other essential syphilis testing supplies found to be unavailable were pipette tips (14%) and gloves (17%) [32].

When asked about length of stock-outs of reagents needed to perform syphilis tests, the mean length of stock-outs was longest for TPHA test (mean 182 days, range 30 to 365 days), followed by the FTA test (mean 140 days, range 21–365 days), then the

RPR test (mean 125 days, range 10 to 365 days) and the EIA test (mean 107 days, range 7¬ to 365 days). RPR reagents and gloves were also commonly reported to be out of stock (mean 142 days for RPR card and 99 days for gloves) (Table 10.3). These results suggest availability of these reagents and supplies may not be monitored systematically and logistics data used to forecast needs may be lacking at some participating laboratories.

Table 10.3: Average Length of Stock Out for Various Types of Reagent and Supplies

Type	Overall		ANC clinic		STI clinic		HIV clinic	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
RPR	125	[10–365]	125	[10–365]	136	[10–365]	125	[10–365]
VDRL	46	[10–123]	46	[0–123]	48	[10–23]	44	[10–123]
TP-PA	89	[2–365]	42	[2–90]	89	[2–265]	172	[2–365]
TP-HA	182	[30–365]	206	[30–365]	155	[30–365]	253	[30–265]
FTA	140	[21–365]	140	[21–365]	140	[21–365]	140	[21–365]
EIA	107	[7–365]	133	[7–365]	56	[7–124]	56	[7–124]
CIA	0	[0]	0	[0]	0	[0]	0	[0]
Rapid Treponemal Tests	75	[15–241]	78	[15–241]	75	[15–241]	75	[15–241]
RPR cards	142	[10–365]	11	[10–365]	135	[10–365]	124	[10–365]
Pipettes	60	[30–120]	60	[30–120]	70	[30–120]	70	[30–120]
Gloves	99	[8–300]	99	[8–300]	123	[8–300]	123	[8–300]

When surveyed laboratories were asked about funding sources, 43% of laboratories reported receiving funding from a national STI program or integrated STI/HIV program in their countries, whereas 33% were funded by local provincial programs and 26% by the national HIV program. However, funding sources for syphilis testing varied substantially by sub-region (Table 10.4).

Table 10.4: Key Funding Sources for Syphilis Testing (n = 69)

	Overall (n=69)	North n=2)	Central (n=22)	Caribbean (n=15)	Andean (n=16)	South Cone (n=14)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Maternal and Child health national program funds	15 (22)	0 (0)	3 (14)	3 (20)	6 (38)	3 (21)
National HIV program funds	18 (26)	0 (0)	4 (18)	3 (20)	7 (44)	4 (29)
National STI program or integrated STI/HIV program funds	30 (43)	1 (50)	9 (41)	4 (27)	10 (63)	6 (43)
Reproductive health program funds	8 (12)	0 (0)	3 (14)	1 (7)	3 (19)	1 (7)
Local (institutions' own) program funds	23 (33)	1 (50)	9 (41)	4 (27)	2 (13)	7 (50)
Special funds available to the national	7 (10)	0 (0)	4 (18)	0 (0)	1 (6)	2 (14)
Donor funds	11 (16)	0 (0)	5 (23)	4 (27)	1 (6)	1 (7)
None – There are no special funds for syphilis testing, the patient must pay out of pocket or through her insurance plan	9 (13)	0 (0)	3 (14)	2 (13)	3 (19)	1 (7)
Government/MOH funds	13 (19)	0 (0)	6 (27)	5 (33)	1 (6)	1 (7)

XI. Conclusions

This survey of syphilis laboratory testing practices represents reports from a convenience sample of national and regional reference laboratories and larger clinical laboratories with a high syphilis case load. We cannot be certain about the generalizability of data reported; however, there was high participation among national reference laboratories across the Region. It was unexpected to find that very few laboratories (n=3), including national/regional reference laboratories, employed direct detection methods that allow confirmation of syphilis through demonstration of T. pallidum. Direct detection tests are helpful in confirming presence of T. pallidum, but require skilled technicians who are experienced in the techniques and lesion material which can be difficult to obtain. Regarding serologic testing, most (77%) surveyed laboratories used both screening and confirmatory testing, predominantly with a traditional algorithm (screening with a non-treponemal test and confirming with a treponemal test). However, 14% of the participating laboratories used only one serology test to diagnose syphilis (i.e., did not employ confirmatory testing), which was also an unexpected finding. Syphilis is a common infection in all countries, and national and regional reference laboratories and large clinical laboratories should be supported in having appropriate diagnostics including both treponemal and quantitative non-treponemal serologic tests (at least one test type for each). If feasible, reference laboratories should have access to and staff trained in direct detection methods. If these tests are not available, countries should be supported to identifying regional options to support future access to these basic syphilis diagnostics.

Relatively few laboratories had adopted POC syphilis testing. Among the reported challenges to introducing RSTs was the lack of appropriate algorithms to support these. Although POC tests are more costly than laboratory-based syphilis test on a per-test basis, rapid tests can be highly cost-effective in settings where treatment coverage is low (i.e., patient loss to follow up high). This is particularly important to consider in situations (e.g., pregnancy) in which prompt treatment is critical to avoid serious, adverse health outcomes. Ultimately, the most expensive laboratory testing situations are when tests are done and results are positive, but cannot be acted upon. If patients are tested but not treated there are costs to the program with no outcomes averted. Thus, POC tests can be comparatively cost-effective in certain situations, such as ANC clinics experiencing low coverage of treatment. In this survey, some laboratories reported being able to turnaround results rapidly (e.g., 4 to 5 hours). However, even this relatively short turnaround time may not be quick enough as it is unlikely that most ANC patients remain at the clinic for 4 hours. The survey results indicated that only seven of the 54 participating laboratories supporting ANC programs used syphilis testing algorithm including RSTs. This is an area that could be explored by local programs.

Many laboratories faced challenges in ensuring adequate supplies to carry out syphilis testing. Certain commodities and equipment are critical to accurate testing, such as reagents that are not expired, new RPR cards, and specialized slides for CSF testing. These should be included as part of essential equipment and commodities projections and purchases. It is important that managers or logistics staff in charge of procurement understand the types of reagents and equipment required for accurate testing, and adequately forecast needs. At the end of the day, inadequate or low quality reagents and lack of appropriate equipment (e.g., new RPR cards, calibrated rotators) lead to inaccurate test results, which are associated with a greater cost than would have been expended with appropriate quality reagents, equipment and supplies. Local re-evaluation of essential commodities procurement regulations and bulk procurement mechanisms could help alleviate stock-outs and better assure accurate supplies and equipment for syphilis testing.

PAHO is working to address the possibility of regional bulk purchasing mechanisms that may lower costs to laboratories. The survey results suggest that other options to lower costs could also be explored. For example, some laboratories continue to use the FTA-ABS, a type of treponemal test that requires extensive technician time and attention. Other types of treponemal tests which require less technician time and expertise would likely reduce costs.

Another important survey result was the finding that some basic practices to ensure quality of syphilis testing were often not performed. An important component of quality assurance is adequately trained technicians; however, lack of opportunities for staff training or refresher courses was commonly reported by laboratories. Additionally, many laboratories did not employ standard procedures such as use of daily controls or routine calibration of equipment. Many used inadequate rotators for serologic testing. Some laboratories reported reusing RPR cards. Quality control and quality assurance mechanisms around syphilis testing should be incorporated as an essential aspect of public health laboratory qualifications.

For reference laboratories, in addition to participation in an external quality assurance program to ensure accurate testing by technicians, there is an implied responsibility of ensuring quality of testing in underlying laboratories in the country or Region. For most countries or sub-regions this would involve the reference laboratory developing a quality assurance plan identifying roles and responsibilities of participating laboratories. Quality of syphilis testing in peripheral laboratories requires a multi-pronged approach including means of ensuring adequate training of staff, wide availability of standard operating procedures, and quality assurance mechanisms. The latter include periodic site visits and observation (ideally as part of an over-arching laboratory quality assurance program); reports from peripheral laboratories on their use of standard operating procedures, human resources and commodities around syphilis testing; and proficiency testing requirements for testing at district and lower level laboratories in both the public and private sectors. If adopted, POC tests also require quality assurance monitoring (e.g. training and refresher courses, periodic observation, proficiency testing program using dried tube specimen (DTS), standard operating procedures or other models). PAHO is exploring web-based training options to support refresher training and ensure standard operating procedures for common tests are available to all laboratories. Other supportive options have been provided by the National Reference Laboratory of Brazil around use of integrated HIV/syphilis DTS for rapid tests, currently being employed to support underlying laboratories throughout the country. This is a model that could be adopted by other nations. The CDC Division of STI Prevention Laboratory Branch, through a collaboration with PAHO and WHO, provides laboratories access to external QA of syphilis testing through a syphilis proficiency testing program that sends panels three times per year to participating laboratories. This program is focused particularly on national reference laboratories, assuming that these reference facilities will provide a national QC model for underlying facilities (as is the case for Brazil).

This survey had some limitations. Many of the reporting laboratories were national or regional reference laboratories, and as noted earlier, their responses may not reflect experience of laboratories at lower health facilities. Not all member states participated, and data are particularly limited from small Caribbean island states. Data were based on the self-report by directors and managers and may not reflect actual practice. Additionally, some critical questions about syphilis testing were either not included in the survey (e.g., use of quantitative non-treponemal tests vs. qualitative tests) or not answered by participating laboratories (e.g., specifics of quality control strategies).

Following the 2014 survey the following steps have already been undertaken: 1) A guidance document on syphilis testing was developed by PAHO with support from CDC, providing examples of algorithms (including rapid testing algorithms) for use in different clinical settings. 2) Reference

laboratories were invited to join external quality assurance (EQA) programs supported by the WHO-PAHO/CDC Collaborating Centre on Syphilis Serologic Testing (Available at: http://www.who.int/reproductivehealth/topics/rtis/syphilis/spt-program/en/). 3) PAHO is developing a website that includes standard operating procedures for common syphilis tests and real-world evaluation reports on marketed rapid syphilis tests, as well as standard operating procedures to integrate proficiency testing for rapid HIV and syphilis diagnostics using dried tube specimens. 4) Future work is planned to support web-based training around syphilis testing and, if possible, bulk procurement mechanisms for the Region and training on forecasting and projecting needs. Additionally, 5) the Brazil Ministry of Health has adapted a DTS EQA program for both HIV/ syphilis rapid tests and is able to support other countries on developing similar models.

In summary, the results of this first-ever survey on syphilis laboratory testing practices indicated that while most countries in LAC conducted basic syphilis testing, the quality of tests and testing may be less than optimal. For several countries, modest changes in syphilis testing practices could lead to large improvements in quality and cost-effectiveness of syphilis testing. Sufficient quality syphilis testing is important for many reasons: Accurate testing can help address the growing syphilis epidemic in MSM which contributes to substantial syphilis-associated morbidity as may contribute to further HIV cases in the Region. Better syphilis testing can help control infections in at-risk, heterosexual populations (e.g., mobile populations). Importantly, prompt, affordable and accurate syphilis testing is important in ensuring control and, ultimately, elimination of congenital syphilis, a regional public health priority on which the Americas is a global leader (6,9).

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