

Canadian Public Health Laboratory Network laboratory guidelines for the use of point-of-care tests for the diagnosis of syphilis in Canada

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AE Singh, MA Chernesky, M Morshed, T Wong. Canadian Public Health Laboratory Network laboratory guidelines for the use of point-of-care tests for the diagnosis of syphilis in Canada. *Can J Infect Dis Med Microbiol* 2015;26(Suppl A):29A-32A.

Syphilis point-of-care tests (POCT) are widely available in developing countries enabling early diagnosis, treatment and support. The majority of commercially available tests use treponemal antigens and the presence of antibodies does not distinguish between current and past infection, which may lead to unnecessary antibiotic use and stigmatization of having a current STI. In hard-to-reach populations, the benefits may outweigh the risks. Available studies show reasonable performance of POCT with median sensitivity of 86%, specificity of 99% and positive predictive values >80% when prevalence was >0.3%. Although no syphilis POCT are approved in Canada at this time, a single study in an outreach setting in Alberta showed limited benefit due to a high prevalence of previous infection but more studies are needed. Newer dual tests employing treponemal and non-treponemal antigens look promising.

Key Words: *Canada; Dual tests; Point-of-care tests; Syphilis; Treponemal*

Les directives du Réseau des laboratoires de santé publique du Canada sur l'utilisation des tests au point de service pour diagnostiquer la syphilis au Canada

Les tests au point de service (TPdS) de la syphilis sont largement répandus dans les pays en voie de développement, ce qui favorise un diagnostic, un traitement et un soutien rapides. La majorité des tests offerts sur le marché font appel aux antigènes tréponémiques. Toutefois, la présence d'anticorps ne permet pas de distinguer une infection en cours d'une infection antérieure, ce qui peut entraîner l'utilisation inutile d'antibiotiques et une stigmatisation liée à l'ITS. Dans des populations difficiles à joindre, les avantages dépassent peut-être les risques. Selon les études existantes, les TPdS donnent des résultats raisonnables, à la sensibilité médiane de 86 %, à la spécificité de 99 % et aux valeurs prédictives positives de plus de 80 % lorsque la prévalence est supérieure à 0,3 %. Même si aucun TDdS de la syphilis n'est approuvé au Canada, une seule étude, réalisée dans un milieu communautaire en Alberta, en a démontré les avantages limités en raison de la forte prévalence d'infection antérieure, mais d'autres études s'imposent. De nouveaux doubles tests, faisant appel à des antigènes tréponémiques et non tréponémiques, semblent prometteurs.

DEFINITION OF A POINT OF CARE TEST

A point-of-care test (POCT) is a test that can be performed outside of a laboratory setting for which the result is available without reference to a laboratory and rapid enough to affect immediate patient management (1). Most POCT for syphilis are available with a short turn-around time with test results available in ≤20 min (2). For developing countries, the tests should be affordable, sensitive, specific, user friendly or simple to perform with minimal training, rapid, robust (ie, stable and not requiring cold-chain storage conditions), equipment-free and delivered to those who need it (www.who.int/std_diagnostics/about_SDI/priorities.htm).

RATIONALE FOR USING POCT FOR SYPHILIS IN CANADA

There is often a delay between testing and treatment even in compliant patients with positive (non POCT) results, during which infection can spread (3-5). Patients' not returning for results of testing for a sexually transmitted infection (STI) is a common problem (3,6-8).

Syphilis POCT are widely available for use in developing countries where they allow people to access a STI test at an earlier time point in their disease; they empower patients by allowing them to take greater responsibility for their own sexual health and expand the settings in which STI testing can be undertaken, enabling earlier diagnosis and access to rapid treatment and support (2,9).

Over the past several years, Canada has experienced syphilis outbreaks in street-involved persons, bathhouses and drop-in centres which are traditionally hard to reach through standard services. POCT would provide the ability to offer immediate testing and treatment in a single encounter to mitigate further spread, and an attractive alternative to standard testing (9,10).

Currently, there are no POCT approved for the diagnosis of syphilis in Canada. The majority of commercially available POCT are based on treponemal antigens. These tests cannot distinguish previously treated infections from untreated syphilis (11). Management based on treponemal antigen based POCT may result in unnecessary administration of antibiotics to patients and may also be psychologically detrimental to patients due to the stigma of a STI diagnosis (10). However, in hard-to-reach populations, the benefit of POCT could potentially outweigh the risks. Recognizing this trade-off, the U.S. Centers for Disease Control and Prevention recommends rapid screening and treatment for patients having positive tests at the first prenatal visit in populations in which use of "prenatal care is not optimal" (12). The United States FDA announced approval of a rapid syphilis test in September, 2011 (13). Syphilis POCT may provide 'while you wait' test results which can be confirmed by a non-treponemal test to identify high-risk patients for improved follow-up.

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TABLE 1
Treponemal tests for detection of syphilis antibodies

| Test | Test name | Manufacturer | |
|----------------------|------------------------------------|--|--------------|
| Point of care | Determine TP | Abbott Laboratories, USA | |
| | Dual Syphilis POC Test | ChemBio Diagnostic Systems, USA | |
| | Espline TP | Fujirebio, Japan | |
| | Guardian One Step | Test Medica Diagnostics, USA | |
| | Rapid Syphilis Test | Quorum Diagnostic, Canada | |
| | SD Bioline 3.0 | Standard Diagnostics, Korea and Pacific Biotech, Thailand | |
| | Syphilis Fast | Diesse Diagnostic, Italy | |
| | Syphilis OnSite Rapid Screening | CTK Biotech, USA | |
| | Syphilis Ultra Rapid | Acon, China | |
| | Syphicheck WB | QualPro Diagnostics, India | |
| | Trep-Strip IV | Phoenix Biotech, Canada | |
| | Visitect Syphilis | Omega Diagnostics, UK | |
| | Laboratory | Bioplex Syphilis | Bio-Rad, USA |
| | | Bioplex 2200 Syphilis IgG | Bio-Rad, USA |
| FTA | | Zeus Scientific, USA | |
| TPPA | | Fujirebio, Japan | |
| TPHA | | Omega Diagnostics, United Kingdom | |
| TrepID | | Phoenix Biotech, Canada | |
| Trep-Check EIA | | Phoenix Biotech, Canada | |
| Trep-Sure EIA | | Phoenix Biotech, Canada | |
| Treponema ViraBlot | | Viramed Biotech AG, Germany | |

TYPES OF POCTS

a) Treponemal tests

Currently (Table 1) there are several commercial tests available internationally (10). They are of two varieties: 1) immunochromatographic strip (ICS) tests which work by having a test strip with a line that is impregnated with treponemal antigens that react with antibodies to syphilis in whole blood or serum to produce a readable change on the test strip; 2) particle agglutination tests (PATs), which use gelatin particles

coated with treponemal antigens that clump together on a test tray when combined with whole blood or serum containing antibodies to syphilis.

The Sexually Transmitted Diseases Diagnostics Initiative (SDI) conducted laboratory based evaluations on seven POCT (11). Tables 2 and 3 summarize the use of POCT in antenatal and other clinical settings. Although some of the studies reported low sensitivity values, the median sensitivity was 86% (interquartile range [IQR] 75% to 94%) and was comparable between antenatal and non-antenatal clinic sites. Two studies showed better sensitivity when serum specimens were used (11,14). Specificities ranged from 91% to 100% for studies with similar medians of 99% (IQR 97% to 100%) in all settings. The POCT also showed good positive predictive values of >80% when syphilis prevalence was >0.3%. Limited data are available to confirm if the sensitivity is maintained in HIV-infected individuals (15) and in those with high RPR titres (15,16). An Australian laboratory based study of four syphilis POCT reported that the Determine test had the highest overall sensitivity with significantly higher test sensitivities among high-RPR titre (RPR \geq 1:8) tests (17).

Only one test, the Syphilis Health Check (Trinity Biotech, USA), is United States FDA approved for use in the United States (18). This 10 min test can be used with whole blood, serum or plasma specimens, requires 25 μ L to 50 μ L of blood. According to the manufacturer, this treponemal POC test for syphilis has a reported 95.6% positive agreement and a 90.5% negative agreement with gold-standard testing, with a percent overall agreement of 90.6% (19). No published clinical data on test performance are available.

In the only published study of syphilis POCT in Canada, Bergman et al (20) reported a sensitivity of 85.3% (CI 68.9% to 95.0%), specificity of 100.0% (CI 99.6% to 100.0%), positive predictive value (PPV) of 100.0% (CI 88.1% to 100.0%), and negative predictive value (NPV) of 99.5% (CI 98.9% to 99.8%) of the SD Bioline 3.0 Syphilis Test (Standard Diagnostics, Korea) in hard-to-reach outreach settings in Edmonton, Alberta (20).

Available data on the antenatal cost-effectiveness of RPOCT show that the ICS TT tests are cost-effective for the detection of maternal syphilis in low resource settings when compared to either standard two-test testing algorithms (ie, NTT followed by TT) or a NTT alone (21-23). Owusu-Edusei et al (24) recently reported that a screening strategy employing an ICS TT was more cost saving than a dual-RPOCT (TT

TABLE 2

Selected studies of syphilis point-of-care tests in antenatal clinics (adapted from Tucker, 2010)

| First author (reference), year (sample) | Location, study population | Test name | Reference standard test | Samples, n | Sensitivity, % | | Specificity, % | | Syphilis antibody prevalence |
|--|--|------------------------|------------------------------|------------|----------------|-------------|----------------|-------|---------------------------------|
| | | | | | (95% CI) | (95% CI) | | | |
| Bronzan (27), 2007 (finger prick) | South Africa, 8 rural clinics | Determine | RPR, TPHA | 341 | 86 (57-98) | 91 (87-94) | | 6.5 | |
| Hernandez-Trejo (28), 2006 (serum) | Mexico, 2 urban clinics | Determine | VDRL, FTA-Abs | 1322 | 100 | 100 | | 0.3 | |
| Lien (29), 2000 (multiple) | Vietnam, one large urban clinic | Determine | RPR, TPPA | 291 | 100 | 99 | | 24.7 | |
| Mabey (30), 2006 (multiple) | Tanzania, one large government clinic | Determine | 2 rapid tests compared with | 528 | 60 (47-72) | 99 (99-100) | | 10.8 | |
| | | Visitectoline | treponemal tests (TPPA, etc) | 528 | 75 (64-86) | 99 (99-100) | | 10.8 | |
| | | Syphicheck-WB | | 582 | 79 (68-89) | 99 (98-100) | | 9.45 | |
| | | SD Bioline 3.0 | | 582 | 86 (77-94) | 98 (97-99) | | 11.34 | |
| Montoya (15), 2006 (finger prick) | Mozambique, 6 rural clinics | SD Bioline 3.0 | RPR, TPHA | 326 | 86 (82-89) | 97 (96-97) | | 8.35 | |
| Tinajeros (31), 2006 (finger prick) | Bolivia, 4 large urban clinics | Determine | RPR, TPPA | 8892 | 92 (88-94) | 98 (98-99) | | 3.85 | |
| Villazon-Vargas (32), 2009 (whole blood; not finger prick) | Bolivia, 1 urban clinic | Determine | RPR, FTA-Abs | 489 | 98 | 100 | | 4.5 | |
| West (33), 2002 (serum) | Gambia, 1 rural clinic | Rapid Syphilis Test | RPR, TPHA | 1325 | 75 | 95 | | 3.0 | |

RPR Rapid plasma reagin; TPPA Treponema pallidum particle agglutination; TPHA Treponema pallidum haemagglutination test; FTA-Abs Fluorescent antibody absorbed; VDRL Venereal disease research laboratory; FSW Female sex workers

TABLE 3

Selected studies of point-of-care tests in non-antenatal settings (adapted from Tucker, 2010)

| First author (reference), year (sample) | Location; study population | Test name | Reference standard test | Samples, n | Sensitivity, % (95% CI) | Specificity, % (95% CI) | Syphilis antibody prevalence |
|--|--|--|---|--------------------------|--|--|---------------------------------|
| Benzaken (16), 2008 (finger prick) | Brazil, urban area and red-light clinic | Visitect | FTA-Abs VDRL | 506 | 57 (46–67) | 99 (97–100) | 17.9 |
| Campos (26), 2006 (whole blood) | Peru, Field based (STW) | Determine | RPR, TPHA | 3862 | 55 (40–70) | 99 | 5.1 |
| Castro (25), 2010 (serum) | Georgia Public Health and clinic samples | Dual POC Test | RPR, TPHA | 1601 | 96 | 95 | 62.9 |
| Gianino (34), 2007 (whole blood; not finger prick) | Italy, one urban clinic | Determine | TPPA or other trep test | 316 | 95 (89–98) | 98 (95–99) | 31.3 |
| Herring (11), 2006 (archived serum) | Worldwide | Determine Syphilis-Fast Espline Syphicheck-WB SD Bioline 3.0 Visitect Syphilis OnSite Rapid | TPPA, TPHA | 800 | 99 (95–98) 86 (82–89) 98 (96–99) 84 (80–88) 95 (92–99) 95 (92–99) 92 (90–95) 85 (81–88) 96 (93–99) | 94 (92–96) 93 (90–95) 93 (90–96) 97 (96–99) 93 (91–94) 95 (92–97) 97 (95–99) 98 (96–99) 95 (92–99) | 50 |
| Mabey (30), 2006 (multiple) | Brazil, one urban clinic | Determine Visitect Syphicheck-WB SD Bioline 3.0 | 2 rapid tests compared with treponemal test (TPPA, TPHA, etc) | 247 244 542 542 | 89 (80–97) 96 (90–100) 84 (74–94) 88 (79–97) | 98 (96–100) 99 (97–100) 100 (99–100) 99 (99–100) | 21.1 20.9 9.2 9.2 |
| Mabey (30), 2006 (multiple) | Haiti, one urban clinic | Determine Visitect Syphicheck-WB SD Bioline 3.0 | 2 rapid tests compared with treponemal test (TPPA, TPHA, etc) | 761 516 543 515 | 73 (59–86) 73 (61–85) 81 (68–93) 100 (n=30) | 99 (98–99) 99 (98–100) 98 (97–99) 98 (97–100) | 5.3 10.7 7.6 5.8 |
| Nessa (35), 2008 (whole blood; not finger prick) | Dhaka, Bangladesh, urban clinics | Syphilis UltraRapid | RPR, TPHA | 648 | 94 | 93 | 20.8 |
| Siedner (14), 2004 (multiple) | San Francisco, CA, USA, one urban clinic | Determine Guardian Trep-Strip IV | TPPA | 127 116 71 | 88 (81–96) 72 (60–84) 70 (54–85) | 100 100 100 | 52.5 |
| Bergman (20), 2013 | Edmonton, Alberta, outreach settings | SD Bioline 3.0 Syphilis Test | Syphilis EIA, RPR and Syphilis InnoLia | 1265 | 85.3 (68.9–95.0) | 100 (99.6–100) | |

CA California; FSW Female sex workers; FTA-Abs Fluorescent antibody absorbed; RPR Rapid plasma reagin; TPPA *Treponema pallidum* particle agglutination; TPHA *Treponema pallidum* haemagglutination test; VDRL Venereal disease research laboratory

and NTT) strategy in a high-prevalence setting but that the dual-RPOCT strategy may significantly reduce overtreatment. No cost effectiveness data are available for developed countries.

b) Non-treponemal tests

Because positive treponemal POCT may indicate new or old infections, a quantitative non-treponemal test is often helpful. However, there are no commercially available non-treponemal POC tests available as a single test at this point.

c) Dual tests

Two commercially available dual tests are currently available. Castro et al (25) evaluated a novel POCT (Chembio Diagnostics System Inc, USA) for the simultaneous detection of non-treponemal and treponemal antibodies in sera of 1601 patients. Results from the dual test were compared with rapid plasma reagin (RPR) and *Treponema pallidum* particle agglutination (TPPA) tests. When compared with the RPR, the reactive concordance of the non-treponemal result was 98.4% when the RPR was $\geq 1:2$. However, when the RPR was ≤ 1 , the sensitivity declined to 88%. When compared to the TPPA, the reactive and non-reactive concordance of the treponemal line was 96.5% and 95.5%. This dual POCT is designed for use with serum, plasma and whole blood. Span Diagnostics (India) also makes a dual test (www.span.co.in/#) but no published data on its performance in the field are available.

DIFFICULTIES ENCOUNTERED WITH POCT

Choice of test kit and specimen type are important when deciding which kit will perform optimally in any given field setting. For example, Campos et al (26) reported lower sensitivities with whole blood (finger-prick) specimens which might have been due to inadequate lighting, lack of use of heparinized capillary tubes for collection of whole blood, false negatives due to previously treated syphilis and a low proportion of samples reactive at low titres. Herring et al (11) showed variability between test lots, day to day testing and differences between testers.

Because POCT are often performed by inexperienced non-laboratorians outside of a laboratory, results can be variable. Judgment is used on subjective interpretation of a band being positive or negative in an ICS or agglutination strength in a PAT. Sufficient lighting should be provided to read results.

Programs may wish to develop procedural manuals in conjunction with a local reference laboratory to include a control and proficiency testing program. This is to ensure the competence of the testing staff as well as the integrity of the testing materials.

Some components of the QA program could include photographs of positive and negative reactions, the running of positive and negative controls, eg, with each new box of kits that is opened; the results should be recorded and logged. Storage conditions for the kits should be specified with logs kept for temperature control and logs kept as

well as procedures developed to respond to incidents, eg, actions for invalid tests, parallel testing discrepancy and control failure.

USE OF POCT FOR THE DIAGNOSIS OF SYPHILIS

It should be noted that similar to other screening tests for syphilis, a single POCT for syphilis may not be adequate for the diagnosis of syphilis and should follow recommended testing algorithms as described in the Chapter on Serologic Testing for Syphilis.

SUMMARY

Although not yet licensed or routinely available in Canada, syphilis POCT have the potential to provide immediate and rapid access to

testing and therefore treatment in 'hard-to-reach populations' or in non-traditional venues to mitigate the spread of syphilis. POCT which employ treponemal tests are also most likely to be of benefit in areas with high prevalence of new syphilis infections together with low rates of previous infection with syphilis. Further studies are needed to evaluate the utility, acceptance, effectiveness, quality control/quality assurance, potential adverse events and cost-effectiveness of syphilis POCT in clinics and field-based settings. Laboratories and clinicians should ensure the development of effective algorithms to confirm cases as well as maintain acceptable quality of POCT.

DISCLOSURES: The authors have no conflicts of interest to declare.

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