Connecticut Department of Public Health Laboratory
STD Testing Services

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Keeping Connecticut Healthy
DPH
Connecticut Department of Public Health

Virology/Serology/STDs Laboratory Section

Phone: 860-920-6662 Fax: 860-920-6661

- Chlamydia/Gonorrhea
- Syphilis
- Herpes
- HIV
- Hepatitis B & C

Trichomonas Vaginalis testing is NOT available at this time

Please fill out a completed Clinical Test Requisition, Form OL-9B, for each Specimen Source/Type submitted.

Client
Authorized Submitter
Street Address
City, State, Zip

♦LAB PROFILE Number:

CLINICAL TEST REQUISITION
STATE OF CONNECTICUT
Dr. Katherine A. Kelley State Public Health Laboratory
395 West Street, Rocky Hill, CT 06067
CLIA ID 07D0644555 / CT License CL-0197
Phone 860-920-6500
CLIENT SERVICES 860-920-6635

ACCESSION LABEL FOR CTDPH LABORATORY USE ONLY

♦ DENOTES REQUIRED INFORMATION

Section 1: Patient Information *(Please Print Clearly)*

♦ Name (Last, First, M.I.) or Identifier:

♦ Street Address: | ♦ City, State, Zip:

♦ Date of Birth: | Gender: ☐ Female ☐ Male ☐ Unknown | Home Phone:

Race (check all that apply): (♦ Race/Ethnicity Information is Required for Blood Lead)
☐ White ☐ Black/African Amer. ☐ Asian ☐ Amer. Indian/Alaska Nat. ☐ Nat. Hawaiian/Other Pacific Islander ☐ Other ☐ Unknown
Ethnicity: ☐ Hispanic ☐ Non-Hispanic ☐ Unknown

Ordering Healthcare Provider: | Phone:

Section 2: Specimen Information

♦ Specimen Storage (Prior to Delivery): ☐ Refrigerated (2-8˚C) ☐ Frozen (<-20˚C) ☐ Ambient Temperature
♦ Specimen Transport/Delivery: ☐ Cold (Ice pack) ☐ Frozen (Dry Ice) ☐ Ambient Temperature

Submitter Sample ID: | ♦ Date Collected: | Time Collected: ☐ AM ☐ PM

♦ Specimen Source/Type:
☐ Blood (whole) ☐ Bronchial Wash ☐ Buccal cavity ☐ Cervix ☐ CSF ☐ Nasopharynx ☐ Oropharynx ☐ Plasma
☐ Rectal ☐ Serum ☐ Sputum ☐ Stool ☐ Urethra ☐ Urine ☐ Vaginal

☐ Body Fluid, specify _____ ☐ Tissue, specify _____
☐ Other, specify

♦ Section 3: Select Testing Requested

Bacteriology

☐ AFB Clinical Specimen (Mycobacteria Smear & Culture)
☐ AFB Referred Culture (Mycobacteria for Identification)
☐ Bioterrorism Agent Identification
specify agent:_____
☐ Bordetella pertussis (DFA, Culture) ☐ (DNA amplification)
☐ Chlamydia/ Gonorrhea Nucleic Acid Amplification Test
☐ CRE panel Organism:_____
☐ EIP Isolates for Identification (Check one)
☐ Group A *Streptococcus* ☐ *H. influenzae* ☐ *L. monocytogenes*
☐ *N. meningitidis* ☐ *S. pneumoniae* ☐ Other: _____
☐ Enteric Isolate for Identification
☐ *Campylobacter* ☐ *E. coli O157* ☐ *Salmonella* ☐ *Shigella*
☐ *Shiga-toxin producing E. coli* ☐ *Vibrio* ☐ Other: _____
☐ Enteric (Stool) Culture ☐ CIDT Organism: _____
☐ Shiga-toxin (+) Broth Culture

Bacterial Serology

☐ QuantiFeron-TB Test (Specify ♦ Date & Time Collected Above)
☐ Syphilis Screen (VDRL)
☐ Syphilis Confirmation (VDRL & TP-PA)
☐ Syphilis CSF (VDRL Only)

Blood Lead (Uninsured Patients ONLY) ♦ Race/Ethnicity Required

☐ Child Lead Screen (Capillary Blood)
☐ Lead Confirmation (Venous Blood)

Mycology

☐ *Candida auris* identification

Parasitology

☐ Blood Parasite - Smear

Virology

☐ Arbovirus IgG/IgM (Encephalitis Viruses)
California Group, Eastern Equine, St. Louis, Western Equine
☐ Hepatitis B Surface Antibody
☐ Hepatitis B Surface Antigen
☐ Hepatitis C Testing
☐ Herpes Simplex IgG Antibody
☐ Herpes Simplex DNA amplification
☐ HIV-1/HIV-2 Ag/Ab
☐ HIV Viral Load
☐ Influenza PCR
☐ Measles PCR
☐ MERS CoV (Novel Coronavirus) (Epidemiology Approval Required)
☐ Mumps PCR
☐ Norovirus PCR (Epidemiology Approval Required)
☐ Respiratory Virus Antigen Panel: *Adenovirus, Human Metapneumovirus, Parainfluenza, Rhinovirus/Enterovirus, RSV*
☐ Varicella Zoster IgG Antibody
☐ West Nile Virus IgM Antibody
☐ Virus Identification (Tissue Culture)
NOTE: Zika virus testing requires submission of the Zika Virus Clinical Test Requisition
SARS-CoV-2 requires COVID-19 requisition form

Test, Agent or Disease, Not Listed (Specify)

Comments

Form OL-9B Rev. 05/12/2021

Current Testing CT DPH LAB

Chlamydia & Gonorrhea Testing

- **Test Description:** Qualitative detection and differentiation of ribosomal RNA (rRNA) from *Chlamydia trachomatis* and/or *Neisseria* gonorrhoeae in genital, extra genital and urine specimens.
- **Methodology**: Target amplification nucleic acid probe test (Aptima Combo 2 on the Panther System)
- **Intended Use**: As an aid in the diagnosis of chlamydial and gonococcal diseases in symptomatic or asymptomatic individuals.
- **Specimen Types:**
  - Vaginal, Throat & Rectal Swabs (*Aptima Multitest Swab Specimen Collection Kit*)
  - Endocervical and Male Urethral Swabs (*Aptima Unisex Swab Specimen Collection Kit*)
  - Urine (*Aptima Urine Specimen Collection Kit)*
- **Specimen Storage:** Store collected specimen tubes between 4°C to 30°C. Urine samples must be tested within 30 days. Swab specimens must be tested within 60 days.
- **Collection Instructions:** Follow instructions provided with each specific collection kit.
  - Rectal, throat, endocervical & male urethral swabs: clinician-collected ONLY. Vaginal swabs: clinician-collected OR patient-collected (not for home use).
  - Specimen must be collected before the expiration date on the collection kit.

Current Testing CT DPH LAB

Syphilis Testing- Screen & Confirmation

- **Test Description:** Non-treponemal assay for the detection of reagin in serum. Reactive results are tittered to end point and confirmed with a treponemal-specific test
- **Methodology:** VDRL (Venereal Disease Research Laboratory) Slide Flocculation Test
- **Intended Use:** Serologic screen to aid in the diagnosis of primary, secondary or tertiary syphilis and for post-treatment evaluation.

  CSF – To rule out neurosyphilis in patients with neurological symptoms.
- **Specimen Types:** Serum, Cerebrospinal fluid (CSF)
- **Specimen Storage:** Store at 2-8°C. Specimens must be received within 5 days of collection.

Current Testing CT DPH LAB

Syphilis Testing- Screen & Confirmation

- **Test Description:** Detection of *Treponema pallidum* antibodies in human serum or plasma.
- **Methodology:** *Treponema pallidum* Particle Agglutination Assay (TP-PA)
- **Intended Use:** To confirm reactive results of non-treponemal syphilis screening (such as VDRL or RPR); as a diagnostic test in individuals with a nonreactive non-treponemal test result but with symptoms suggestive of late syphilis.
- **Specimen Types:** Serum, Plasma
- **Specimen Storage:** Store at 2-8°C. Specimens must be received within 5 days of collection.

Current Testing CT DPH LAB

Herpes Simple Virus (HSV) DNA Testing

- **Test Description:** Qualitative assay for the detection of Herpes simplex virus 1 and 2 DNA in human specimens.
- **Methodology:** Loop-mediated amplification and detection.
- **Intended Use:** Direct detection and differentiation of herpes simplex virus 1 and 2 to aid in the diagnosis of infection.
- **Specimen Types:** Cutaneous or mucocutaneous lesion swab submitted in viral transport media, 1-3 mL.
- **Specimen Storage:** Store at 2-8°C. Specimens must be received and tested within 7 days of collection.

Current Testing CT DPH LAB

Herpes Simplex Virus IgG Antibody Testing

- **Test Description:** Qualitative assay for the detection of IgG antibodies to herpes simplex virus (HSV) type 1 and type 2 in human serum. This test does not differentiate between type 1 or type 2.
- **Methodology:** Enzyme immunoassay (EIA)
- **Intended Use:** As an indication of past infection with herpes simplex virus to identify asymptomatic carriers.
- **Specimen Types:** Serum
- **Specimen Storage:** Store at 2-8°C. Specimens must be received within 2 days of collection.

Current Testing CT DPH LAB

HIV/Hepatitis Testing

- HIV 1/2 Antibody/Antigen Enzyme Immunoassay (EIA)
- HIV 1 & 2 Antibody Differentiation Assay (BioRad Geenius)
- HIV-1 RNA Viral Load (plasma only)
- Hepatitis C Antibody Enzyme Immunoassay (EIA)
- Hepatitis C RNA Viral Load (serum and plasma)
- Hepatitis B Surface Antibody Enzyme Immunoassay (EIA)
- Hepatitis B Surface Antigen Enzyme Immunoassay (EIA)

State of Connecticut Department of Public Health State Public Health Laboratory Website

- Laboratory Resources:
  - **Collection Supplies and Test Requisition Forms**

    https://portal.ct.gov/DPH/Laboratory/Scientific-Support/Scientific-Support-Services

    Collection supplies may be requested by calling Scientific Support Services at 860-920-6674 for by submitting an email request to dph.outfitroom@ct.gov

  - **Directory of Clinical Testing Services**

    https://portal.ct.gov/DPH/Laboratory/Clinical-Testing-Services/DCTS-101915

What Happens at the Lab

Sample Receiving Area

- Sample Evaluation
- Requisition Evaluation
- Accessioning & Data Entry in LIS
- Delivery to Laboratory for Testing

Testing Laboratory

- Specimen: checked for proper labeling (name on tube must match name on test requisition), volume, proper collection tube for sample type & tube outdate (CT/GC). Serum is aliquoted into transfer tubes and frozen.
- Requisitions checked for errors and omissions.

Testing Laboratory Activity

- Testing, Reporting and Checking Results for Accuracy.
- Quality Assurance Report sent out for test requests with sample or requisition inconsistencies and omissions.
- Testing Not Performed if specimen unlabeled, no specimen or inadequate specimen in tube, wrong collection kit for sample type collected, broken or leaking.
- Positive results:
  - Syphilis VDRL reactive and TPPA reactive results called to submitter
  - All positive HIV results called to submitter
  - CT/GC, Herpes, Hepatitis positive results not called out unless a quality assurance issue needs to be resolved.

Syphilis Serology

The diagnosis of syphilis infection still relies on both clinical evaluation and one of two multitest laboratory testing algorithms to indicate current or past infection with *Treponema pallidum*.

Two types of serological Syphilis tests:

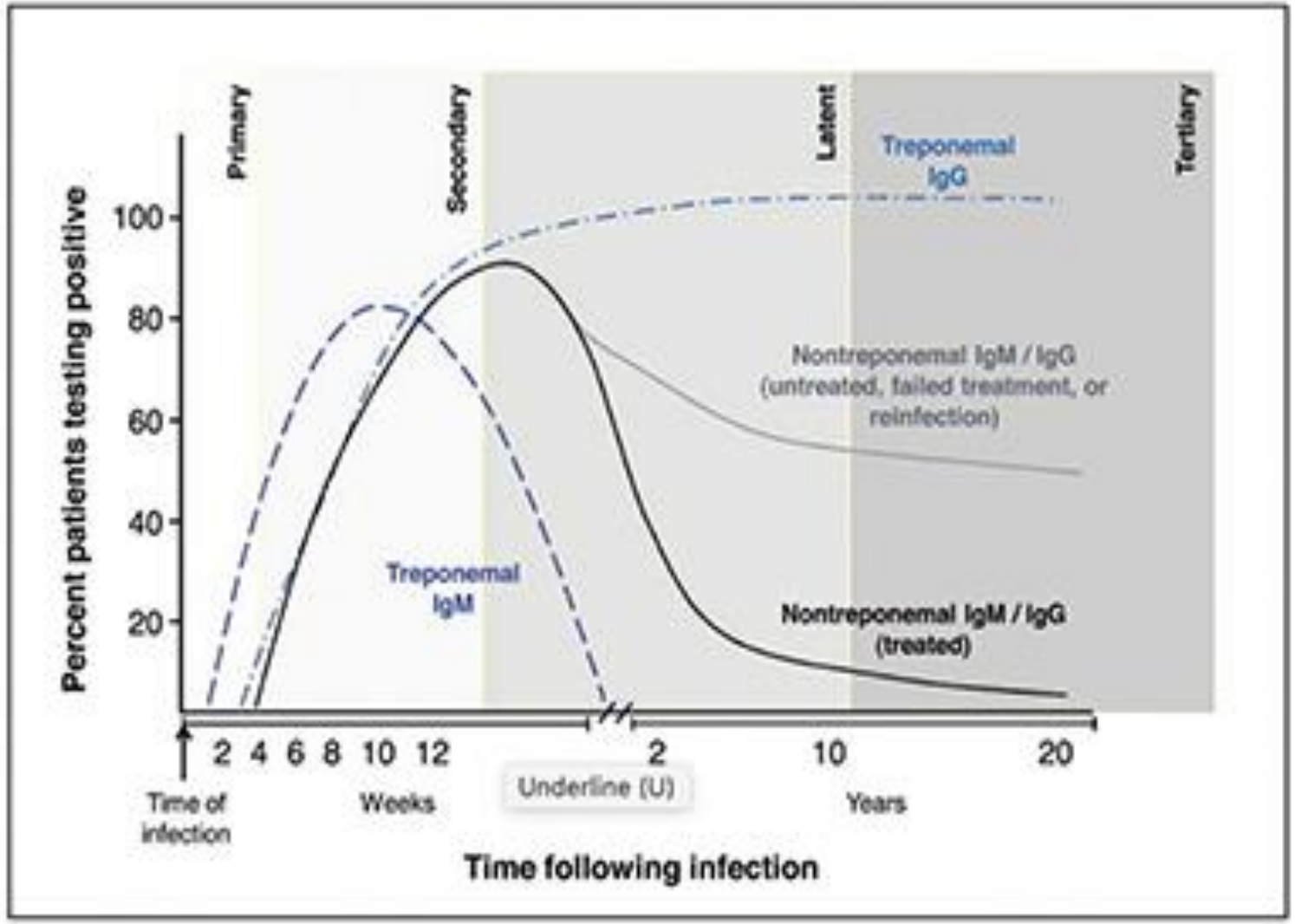
- **Treponemal assays** – detect antibodies to *T. pallidum.*

  Treponemal tests include either IgM, IgG or total (IgG/IgM) antibodies. Antibodies detected by treponemal tests arise earlier than those detected by non-treponemal tests and typically remain detectable for life, even after successful treatment.

- **Nontreponemal assays** – detect antibodies (IgG/IgM) directed against lipoidal antigens (cardiolipin, cholesterol and lecithin), released as a consequence of cell damage, which is elevated in numerous chronic conditions and infections including syphilis.

  Nontreponemal tests can provide an end point titer for positive samples. Titers decline after proper treatment over a period of months to years.

F1 Syphilis Staging and Serology



From Soreng K, Levy R, Fakile Y. Serologic testing for syphilis: Benefits and challenges of a reverse algorithm. Clin Microbiol News 2014;36:195-202.

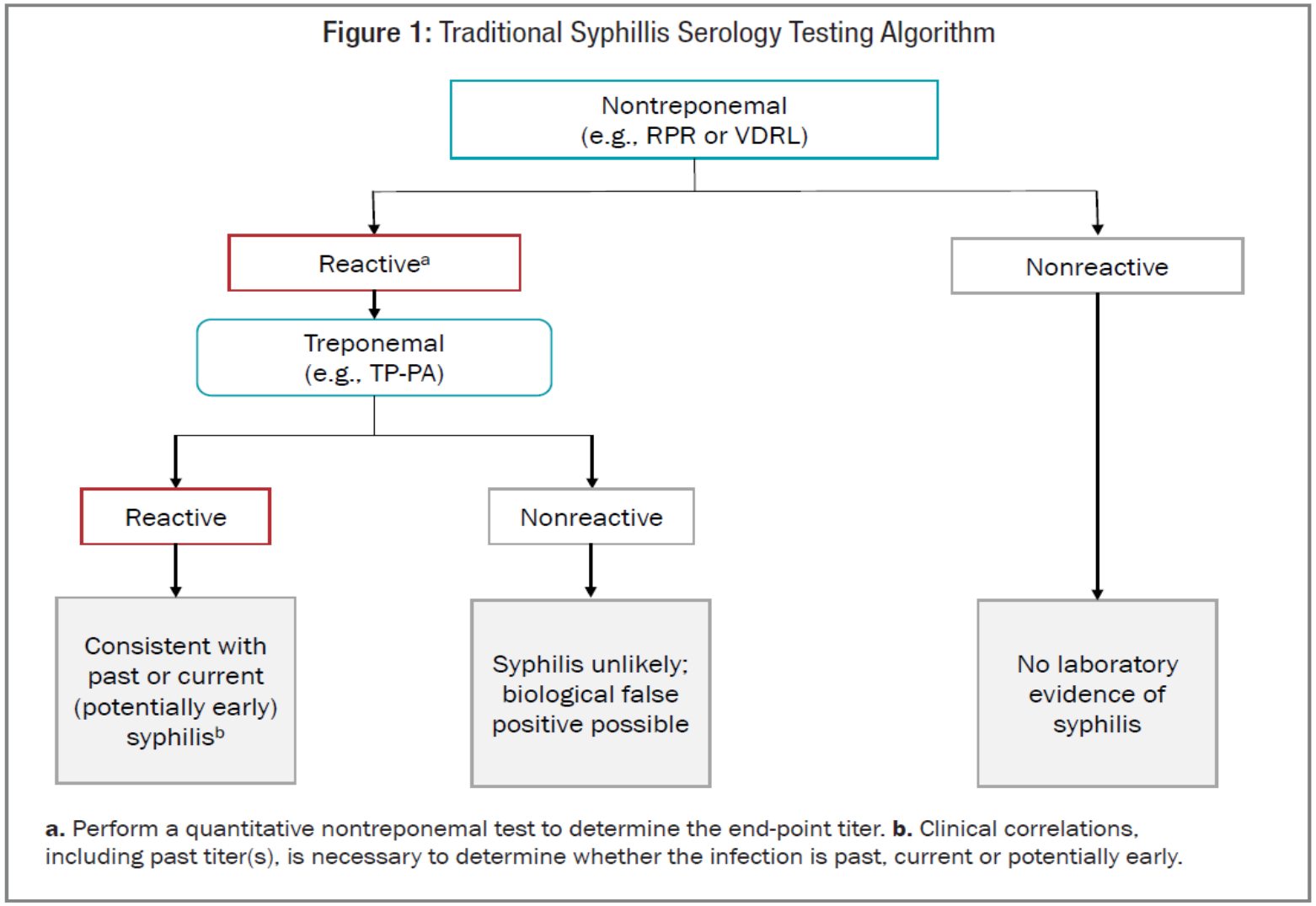
Table 1: Serologic Methods for Syphilis Diagnosis

Method	Manual vs. Automated	Antibodies
Nontreponemal		
Venereal Disease Research Laboratory (VDRL)[a]	Manual	
Rapid plasma reagin (RPR)	Manual or Automated	
Toluidine red unheated serum test (TRUST)	Manual	
Unheated serum reagin (USR)	Manual	
Treponemal		
Fluorescent treponemal antibody absorption (FTA-ABS)[a]	Manual	IgM/IgG
Treponema pallidum particle agglutination (TP-PA) assaya	Manual	IgM/IgG
Line immunoassay (LIA)	Manual	IgG
Enzyme-linked immunoassay (EIA)	Manual/Automated	IgG or IgM/IgG
Chemiluminescent immunoassay/Chemiluminescent microparticle immunoassay (CIA/CMIA)	Automated	IgG or IgM/IgG
Microbead immunoassay (MBIA)	Automated	IgG or IgM/IgG
Rapid antibody test	Manual	IgG

a. These methods may be performed on cerebrospinal fluid (CSF) to support a diagnosis of neurosyphilis. For further information on diagnosis of neurosyphilis please refer to the *Consultation on Laboratory Diagnosis of Syphilis*[10] and the CDC *STD Treatment Guidelines*.[11]

Association of Public Health Laboratories. Suggested Reporting Language for Syphilis Serological Testing-2nd Edition. August 2020

Traditional Syphilis Serology Testing Algorithm



Association of Public Health Laboratories. Suggested Reporting Language for Syphilis Serological Testing-2nd Edition. August 2020

Table 2: Guidance for Reporting Results from the Traditional Syphilis Serology Testing Algorithm performed on Seruma

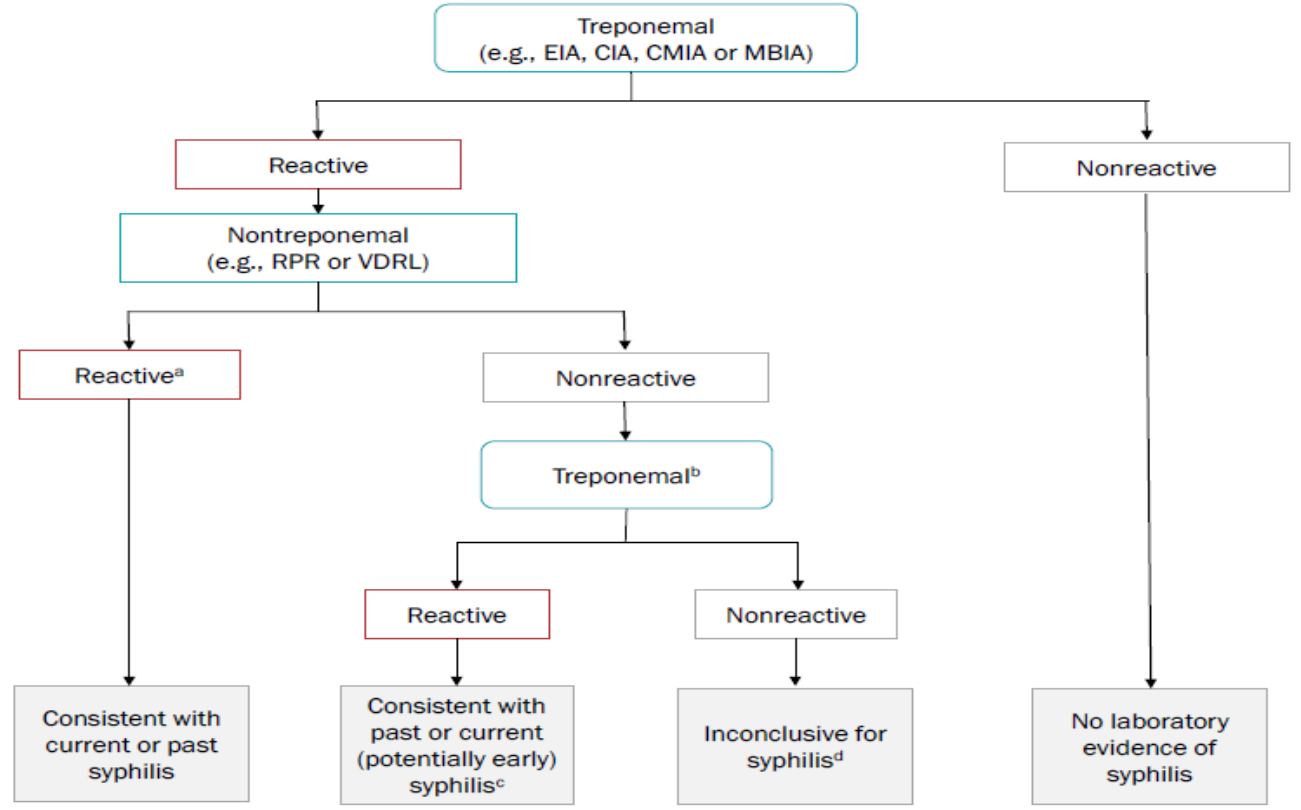
Test Outcomes	Test Sequence			Interpretation for Laboratory Report	Further Actions[c]
	Step 1[a]	Step 1[b]	Step 2		
	Nontreponemal Assay (Qualitative)[b]	Nontreponemal Assay (Quantitative)	Treponemal Assay		
	Nonreactive	Not Indicated	Not Indicated	No laboratory evidence of syphilis	If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.
	Weakly Reactive[d]	Weakly Reactive[de]	Nonreactive	Nontreponemal antibodies detected. Syphilis unlikely; biological false positive possible[f]	Clinical evaluation should be performed to identify signs, symptoms or past history of infection. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.
	Weakly Reactive[d]	Weakly Reactive[de]	Reactive	Treponemal antibodies detected. Consistent with past or current (potential early) syphilis	Clinical evaluation should be performed to identify current signs and symptoms and past history of infection or treatment. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.
	Reactive	Reactive at ≥ 1:1[e]	Nonreactive	Nontreponemal antibodies detected. Syphilis unlikely; biological false positive possible	Clinical evaluation should be performed to identify current signs and symptoms or past history of infection or treatment. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.
	Reactive	Reactive at ≥ 1:1[e]	Reactive	Treponemal and nontreponemal antibodies detected. Consistent with past or current (potential early) syphilis	Clinical evaluation should be performed to identify current signs and symptoms and past history of infection or treatment.
	Special Circumstances: Not recommended in algorithm, for use if both tests are ordered by provider.				
	Nonreactive	Not Indicated	Nonreactive	No laboratory evidence of syphilis	If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.
	Nonreactive	Not Indicated	Reactive	Treponemal antibodies detected. Consistent with past or current (potential early) syphilis	Clinical evaluation should be performed to identify current signs and symptoms and past history of infection or treatment. If past treatment reported, no further management is needed unless recent exposure suspected. If no past history of treatment and recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm

a. This table is for testing and reporting of serum specimens only. b. If the result in nonreactive or weakly reactive, consideration should be given to the possibility of the prozone effect. c. Comments under "Further Action" can be included as language in the laboratory report or can be used as guidance for laboratorians to discuss test results with health care providers. d. A weakly reactive result is a reportable result from VDRL and RPR. e. Refer to package insert for specific reporting language, for certain methods a 1:1 titer may be reported as minimally reactive in certain circumstances. f. For a summary of factors associated with biological false positives review Topic 2, Recommendation 8 of APHL Consultation on Laboratory Diagnosis of Syphilis, Meeting Summary Report.[9]

Association of Public Health Laboratories. Suggested Reporting Language for Syphilis Serological Testing-2nd Edition. August 2020

Reverse Syphilis Serology Testing Algorithm

Figure 2: Reverse Syphillis Serology Testing Algorithm



a. Perform a quantitative nontreponemal test to determine the end-point titer. **b.** The second treponemal test should utilize a unique platform and/or antigen, different than the first treponemal test, commonly a TP-PA is used at this step. Other publications have tables comparing platforms and antigens in treponemal tests.[3,8,11] **c.** Clinical correlation, including past titer(s), is necessary to determine whether the infection is past, cureent or potentially early. **d.** This result could represent an early infection if the first treponemal immunoassay is more sensitive OR false positivity from the first treponemal test.

Association of Public Health Laboratories. Suggested Reporting Language for Syphilis Serological Testing-2nd Edition. August 2020

Table 3: Guidance for Reporting Results from the Reverse Syphilis Serology Testing Algorithm performed on Serum[a]

Test Outcomes	Test Sequence			Interpretation for Laboratory Report	Further Actions[c]
	Step 1	Step 2	Step 3		
	Treponemal Assay	Nontreponemal Assay (Quantitative)[b]	Treponemal Assay		
	Nonreactive	Not Indicated	Not Indicated	No laboratory evidence of syphilis	If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.
	Reactive	Nonreactive	Nonreactive	Treponemal antibodies not confirmed. Inconclusive for syphilis; potential early syphilis, possible false positive	If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm. If clinical suspicion is low no further evaluation is necessary.
	Reactive	Nonreactive	Reactive	Treponemal antibodies detected. Consistent with past or current (potential early) syphilis	Clinical evaluation should be performed to identify current signs and symptoms or past history of infection. If past history of treatment reported, no further management is needed unless symptomatic or recent exposure suspected. If no symptoms or past history of treatment, and if recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm. If results repeat consult with clinician or use the Clinical Consultation Service.
	Reactive	Reactive at ≥ 1:1[d]	Not Indicated	Treponemal and nontreponemal antibodies detected. Consistent with current or past syphilis.	Clinical evaluation should be performed to identify current signs and symptoms or past history of infection.
	Special Circumstances: Not recommended in algorithm, for use if both tests are ordered by provider.				
	Nonreactive	Reactive at ≥1:1[d]	Nonreactive	Nontreponemal antibodies detected. Syphilis unlikely; biological false positive possible.[e]	Clinical evaluation should be performed to identify current signs and symptoms or past history of infection. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm..

a. This table is for testing and reporting of serum specimens only. b. If the result in nonreactive or weakly reactive, consideration should be given to the possibility of the prozone effect. c. Comments under "Further Action" can be included as language in the laboratory report or can be used as guidance for laboratorians to discuss test results with health care providers. d. Refer to package insert for specific reporting language, for certain methods a 1:1 titer may be reported as minimally reactive in certain circumstances. e. For a summary of factors associated with biological false positives review Topic 2, Recommendation 8 of APHL Consultation on Laboratory Diagnosis of Syphilis, Meeting Summary Report.[9]

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Advantages of a Reverse Syphilis Test Algorithm

Advantages of screening with a Treponemal IgM/IgG Immunoassay:

- Specific to Syphilis, nontreponemal assays are relatively nonspecific.
- More sensitive then nontreponemal assays for detecting primary and late latent syphilis.
- Available on automated instruments, less labor intensive.
- Potential for quicker turn-around-time.

Upcoming Changes to STD Testing Services at the CT DPH Lab

- Purchase and Implementation of the Bio-Rad BioPlex 2200 System
- Assays we will be implementing on the new system include:
  - BioPlex 2200 Syphilis Total & RPR Assay
  - BioPlex 2200 HIV Ag-Ab Assay
  - BioPlex 2200 HSV-1 & HSV 2 IgG Assay

The BioPlex 2200 System is a fully-automated, random access, multiplex testing platform for the clinical diagnostic assays.

BioPlex 2200 Syphilis Total & RPR Assay

- **Intended Use:** The BioPlex 2200 Syphilis Total & RPR Assay is a multiplex flow immunoassay utilizing sets of coated magnetic beads, intended for the qualitative detection of total (IgG/IgM) antibodies to *Treponema pallidum* and the qualitative detection and/or titer determination of non-treponemal reagin antibodies in human serum or plasma.
- It is a dual assay that simultaneously detects and differentiates antibodies to *T. pallidum* and reagin antibodies produced during syphilis infection.
- Automates RPR titers up to 1:2,048

BioPlex 2200 Syphilis Total & RPR Assay Limitations and Considerations

- Validated sample types include serum and plasma.

  The CT DPH Lab will need to retain the Syphilis VDRL assay for testing of cerebrospinal fluid (CSF) samples.

- The performance of the BioPlex 2200 Syphilis Total & RPR Assay has not been established on neonates or individuals younger than 7 years old.

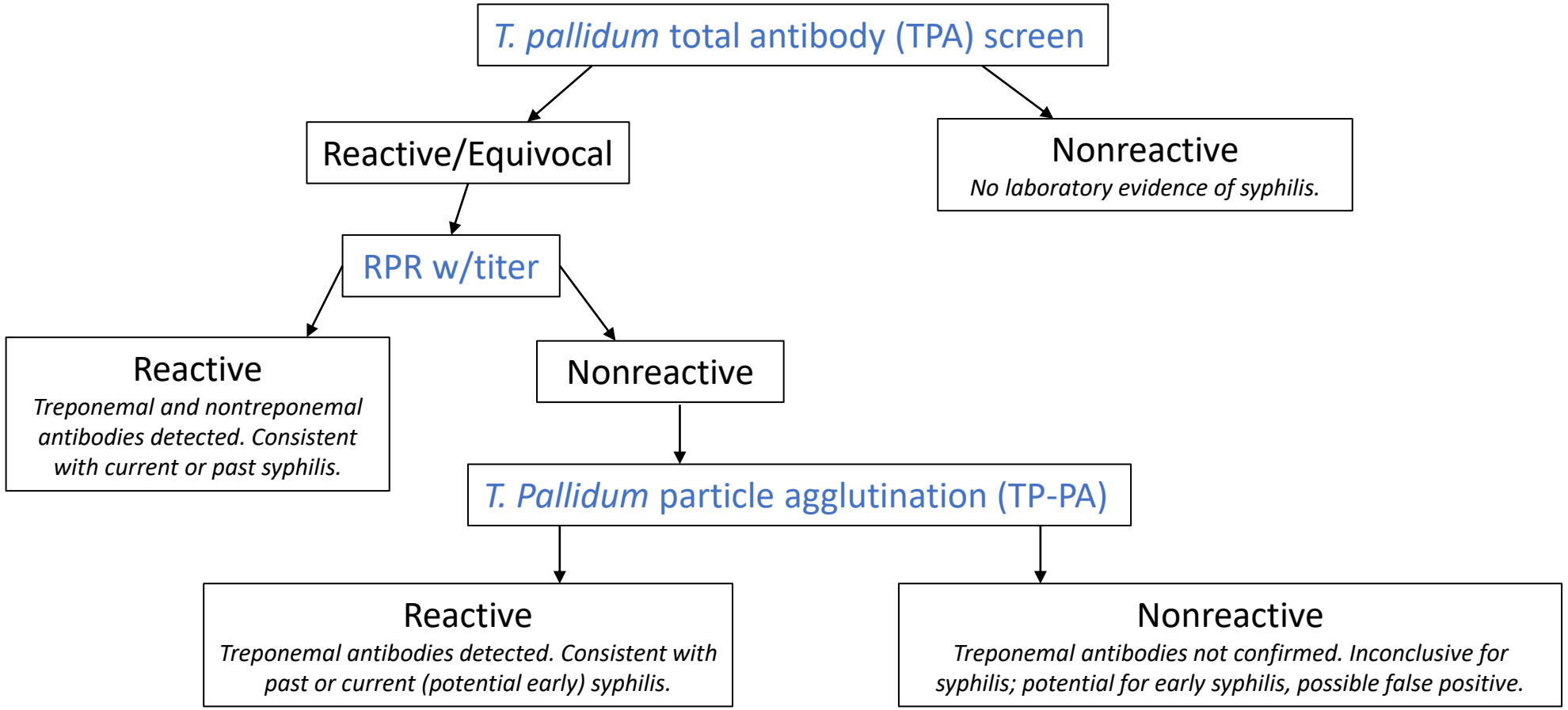
  The CT DPH Lab will need to retain the Syphilis VDRL assay for testing infants.

- It is not recommended to compare BioPlex RPR assay endpoint titers to those generated by manual methods since the manual tests are highly subjective. Higher discrepancy would be observed for cutoff/borderline/weak positive samples.

  When assessing the response to Syphilis treatment, it is recommended to use the same test methodology when comparing titers.

Anticipated Reverse Syphilis Test Algorithm at CT DPH Laboratory

Syphilis Ab w/reflex RPR, serum/plasma only



Anticipated HIV and HSV Test Changes

- The **Bioplex 2200 HIV Ag-Ab assay** will replace the BioRad GS HIV Combo Ag/Ab EIA as the screening test used for the CDC Recommended HIV Testing Algorithm.

  Reports out individual HIV analytes (HIV-1 Ab, HIV-2 Ab and HIV-1 p24 Ag). Supplemental testing will still be performed.

- The **Bioplex 2200 HSV-1 & HSV-2 IgG** assay will replace the current HSV IgG EIA.

  Provides qualitative detection and differentiation of IgG antibodies to HSV-1 & HSV-2.

Next Steps for BioPlex 2200 Testing

- Installation of the BioPlex 2200 - early November 2021
- Training of staff and validation of BioPlex 2200 assays
- SOPs-created and approved by management/QA
- LIMS- Acodes created and tested
- Test Requisition updated
- Directory of Clinical Testing Services – written for each assay and website updated.

  Specimen storage requirements for BioPlex 2200 assays (Syphilis Total & RPR, HIV Ag-Ab, HSV-1 & HSV-2 IgG): serum stored at 2-8°C for up to 7 days.

  Anticipated “go live” date for new testing in 3 to 4 months.

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