Syphilis Testing: Traditional versus Reverse Algorithm

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OBJECTIVES

- Principles of traditional and reverse algorithm
  - Syphilis disease, Syphilis testing, Testing algorithms
- Requirements to report results of individual tests in the algorithm
  - Tests offered by laboratories, What to include in final interpretive statement, Examples
- How to build a syphilis testing algorithm in LIS
  - General guidelines, Reflexed testing – correct billing, Concrete examples of LIS syphilis test builds

2

OBJECTIVES

- Principles of traditional and reverse algorithm
  - Syphilis disease
  - Syphilis testing
  - Testing algorithms

3

DISEASE

- Sexually transmitted disease caused by the spirochete *Treponema pallidum*
- If untreated, the disease progresses to its later stages and eventually to death
- Current antibiotic-based treatment cures affected individual from the disease syphilis but does not repair damage caused by the spirochete

4

DISEASE – STAGES & SYMPTOMS

- **Primary** (contagious)
  - Primary symptom - sores at the site of infection
  - Sores - heal within 3-6 weeks with or without treatment
  - Progression - can be unnoticed, then advances to secondary stage
- **Secondary** (contagious)
  - Primary symptoms - rash on multiple parts of the body and sores
  - Other possible symptoms - fever, swollen lymph glands, sore throat, patchy hair loss, headaches, weight loss, muscle aches, and fatigue
  - Progression – symptoms will disappear with or without treatment, can be unnoticed, advances to latent stage

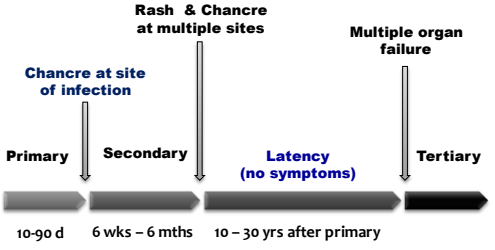
5

DISEASE – STAGES & SYMPTOMS

- **Latent** (usually not contagious)
  - No symptoms
  - Progression - if still untreated, tertiary syphilis will develop in about 1/3 of affected individuals
- **Tertiary** (develops ~10-30 after initial infection, usually not contagious)
  - Symptoms – related to multiple organs being affected: heart, blood vessels, brain, nervous system, internal organs
  - Progression - if still untreated, can result in death

6

UNTREATED SYPHILIS PROGRESSION



7

NEURO & OCULAR SYPHILIS

- **Neurosyphilis**
  - Syphilis that spreads to the brain and nervous system
  - Can develop at any syphilis stage (primary – tertiary)
  - Symptoms - severe headache, difficulty coordinating muscle movements, paralysis, numbness, dementia
- **Ocular Syphilis**
  - Syphilis that spreads to the eye
  - Can develop at any syphilis stage (primary – tertiary)
  - Symptoms – changes in vision to blindness

8

CONGENITAL SYPHILIS

- Syphilis passed by the affected mother to her baby during pregnancy
- Effects of congenital syphilis on pregnancy:
  - Miscarriage, stillbirth, prematurity, low birth weight, death shortly after birth
- Effects of congenital syphilis on the baby:
  - Deformed bones, anemia , jaundice, neurological problems, vision problems, meningitis, skin rashes

9

TESTING CATEGORIES

- **Direct tests**
  - Tests detecting the presence of *T. pallidum* species
  - Methodology - microscopy, histology, molecular testing
  - Not part of algorithms
- **Indirect tests**
  - Two major types:
    - Nontreponemal - detect antibodies against common compounds derived from host cells due to *T. pallidum* infection
    - Treponemal - detect antibodies derived from *T. pallidum* or its components
  - Methodology – serology, immunoassays (CIA, EIA)

10

DIRECT TESTS

- **Dark-Field Microscopy**
  - Detects the presence of viable *T. pallidum* spirochetes in wet mounts by looking for characteristic morphology and motility:
    - 10-14 coils per organism, length of 6-20 micrometers, corkscrew motion
  - Specimen
    - Exudates and fluids from lesions present during primary or secondary stages
  - Advantage
    - Provides immediate definitive diagnosis
    - Rapid

11

DIRECT TESTS

- **Direct Fluorescent Antibody**
  - Detects the presence of *Treponema* species
    - Labeled antibody binds to the antigen found only on pathogenic *Treponema* species.
    - No viable organism is required to be present.
  - Specimen
    - Exudates and fluids from lesions present during primary or secondary stages
  - Advantages
    - Simpler than dark-field
    - Specific for *Treponema* pathogenic species
    - Useful for oral and rectal lesions

12

DIRECT TESTS

Molecular Testing

- Detects the presence of *T. pallidum* in blood
- Specimen
  - Blood
- When useful
  - Congenital syphilis, neurosyphilis, early primary syphilis
- Limitations
  - Very expensive, lack of standardization
- Advantage s
  - Sensitive, detect 1-10 organisms per specimen; very specific for *T. pallidum*

13

INDIRECT TESTS

Nontreponemal Manual Tests - Serology

- Detect the presence of IgG and IgM antibodies against compounds (cardiolipin, cholesterol and lecithin) released from cells due to the presence of *T. pallidum.* (These compounds are not *T. p.* specific, but they are increased in syphilis.)
- Become positive 1-4 weeks after the appearance of primary chancre and 6 weeks after the exposure. Reactivity disappears after treatment.
- Specimen
  - Plasma or serum
- Available tests
  - RPR, VDRL, USR, TRUST

14

INDIRECT TESTS

Nontreponemal Manual Tests - Serology, cont'd

- When Useful
  - Can be used during and after treatment
  - Therapy monitoring (titer changes)
  - Evaluate possible reinfection
- Limitations
  - Lower sensitivity during early primary, late latent and tertiary stages
  - False-positive reactions occur (see table on slide 26)
  - Potential false low reactivity due to prozone effect
- Advantages
  - Rapid, cheap, simple, quantitative, easy to perform
  - Convenient specimen type (plasma/serum)

INDIRECT TESTS

Nontreponemal Manual Tests - Serology, cont'd

VDRL

- Microscopic flocculation slide test read under microscope
- Uses antigen with standardized amounts of cardiolipin, cholesterol and lecithin
- The only test sensitive enough to be used for CSF specimens
- Antigen suspension must be prepared fresh daily

RPR

- Macroscopic flocculation card test
- Simplified version of VDRL
- Uses stabilized VDRL antigen
- Charcoal particles allow for visualization of the reaction
- Most commonly used nontreponemal test

16

VDRL, Veneral Disease Research Laboratory; RPR, Rapid Plasma Reagin

INDIRECT TESTS

Treponemal Manual and Automated Tests – overview

- Detect the presence of antibodies against antigens derived from *Treponema* species.
- Specimen
  - Plasma or serum
- Most common tests
  - FTA-ABS, TP-PA, EIA, Chemiluminiscence

17

INDIRECT TESTS

Treponemal Manual and Automated Tests – overview, cont'd

- When Useful
  - Screening when following reverse algorithm
  - Diagnostic together with nontreponemal tests and clinical symptoms
- Limitations
  - In 75-85% patients tests stay reactive for life – cannot be used to monitor therapy or evaluate reinfection
  - Detect other than T. pallidum species of *Treponema* genus
- Advantages
  - Specific for *Treponema* species

18

INDIRECT TESTS

Treponemal Manual Tests - Serology

- FTA-ABS
  - Fluorescent treponemal antibody absorption test
  - Indirect fluorescence
  - Limitation – variable results due to differences in equipment, reagents and interpretation
- TP-PA
  - *Treponema pallidum* agglutination assay
  - Less expensive and simpler than FTA-ABS
  - One of most commonly used treponemal serological assay

19

False Positive Rate

Causes of False-Positive Reactions in Serologic Tests for Syphilis

Disease	RPR/VDRL	FTA-ABS	TP-PA
Age		Yes	
Autoimmune Diseases	Yes	Yes	
Cardiovascular Disease		Yes	Yes
Dermatologic Diseases	Yes	Yes	--
Drug Abuse	Yes	Yes	
Febrile Illness	Yes		
Glucosamine/chondroitin sulfate		Possibly	
Leprosy	Yes	No	--
Lyme disease		Yes	
Malaria	Yes	No	
Pinta, Yaws	Yes	Yes	Yes
Pregnancy	Yes*		
Recent Immunizations	Yes	--	--
STD other than Syphilis		Yes	

*May cause increase in titer in women previously successfully treated for syphilis

Source: Syphilis Reference Guide, CDC/National Center for Infectious Diseases, 2002

INDIRECT TESTS

Treponemal Automated Tests - Immunochemistry

- Enzyme Immunoassay (EIA)
  - Multiplex bead technology (BioRad BioPlex)
  - High throughput
  - Used as first test in CDC reverse algorithm and in European Centre for Disease Prevention and Control (ECDC) algorithm
- Chemiluminiscence Immunoassay (CIA)
  - Chemiluminiscence format (Advia Centaur)
  - High throughput
  - Used as first test in CDC reverse algorithm

21

ALGORITHMS

WHY DO WE NEED AN ALGORITHM?

A. The only direct diagnostic method that identifies *Treponema pallidum with* 100% certainty is dark-field microscopy.

B. Dark field microscopy is effective only when high number and viable bacteria are present, which occurs only during stages when chancre are present (primary and secondary syphilis).

C. Dark field microscopy thus cannot be used for screening, monitoring therapy, evaluation of reinfections and testing during other stages than the ones when chancre are present. When combined, indirect methods combined can be used in all stages of the disease.

22

ALGORITHMS

WHY DO WE NEED AN ALGORITHM? – cont'd

A. Indirect methods are easy to perform, and with automation, new algorithm (reverse) allows for high throughput screening.

B. Because indirect methods lack specificity of dark field microscopy, at least **two independent indirect tests need to be positive (reactive) for syphilis** before the laboratory tests can confirm the diagnosis.

C. Based on the number of indirect tests laboratory performs, traditional or reverse algorithm is performed.

23

TRADITIONAL VS. REVERSE ALGORITHM

TRADITIONAL

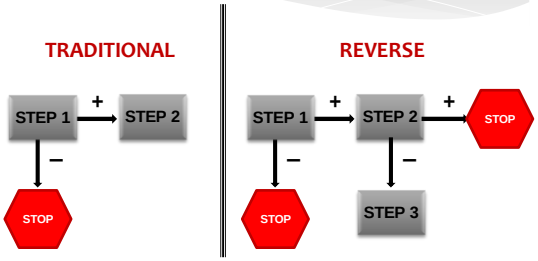
- **2-step** algorithm
- **Step 1:**
  Nontreponemal, QUANTITATIVE assay (manual)
  STOP here when negative
- **Step 2:**
  Only perform when Step 1 test is positive

REVERSE

- **3-step** algorithm
- **Step 1:**
  Treponemal, QUALITATIVE assay (automated)
  STOP here when negative
- **Step 2:**
  Only perform when Step 1 test is positive
  STOP here when positive
- **Step 3:**
  Only perform when Step 2 test is negative

24

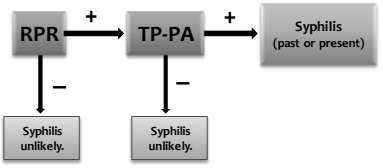
TRADITIONAL VS. REVERSE ALGORITHM



25

TRADITIONAL ALGORITHM

- Begins with manual, nontreponemal, quantitative test



26

TRADITIONAL ALGORITHM

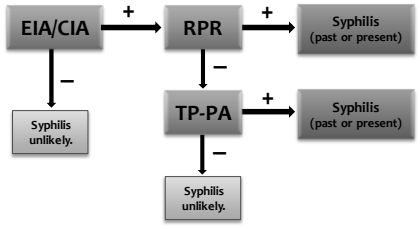
- SUMMARY

- Advantages over reverse algorithm
  - Inexpensive and rapid
  - Detects active infection
  - High positive predictive value
- Disadvantages over reverse algorithm
  - Often misses early or treated infection
  - False negative results due to prozone effect
  - False positives occur with moderately high rate for initial nontreponemal test
  - Needs confirmation by treponemal tests
- Useful in low volume facilities

27

REVERSE ALGORITHM

- Begins with automated, treponemal, qualitative test



REVERSE ALGORITHM

- SUMMARY

- Advantages over traditional algorithm
  - Initial screen starts with automated test – very useful in high volume testing
  - Detects early primary and treated infection
  - No false negatives due to prozone effect
- Disadvantages over traditional algorithm
  - Screening cannot differentiate between active and previously treated infection
  - Requires follow-up nontreponemal test with titer to detect active infection
  - Low risk populations – high false positive rate (neg. RPR, requires confirmation by manual treponemal test)

29

OBJECTIVES

- Requirements to report results of individual tests in the algorithm
  - Tests offered by laboratories
  - What to include in final interpretive statement
  - Examples

30

REQUIREMENTS TO REPORT

TESTS OFFERED BY LABORATORIES

- **Complete Traditional algorithm** – in facilities where automated screening test is not available, occupational workers
- **Complete Reverse algorithm** – in large volume facilities where automated screening test is available

31

REQUIREMENTS TO REPORT

TESTS OFFERED BY LABORATORIES, cont'd

- **Nontreponemal only (RPR, VDRL)** – neonates, after screening with treponemal testing at a different facility, detecting recurrent infections, monitoring treatment
- **VDRL testing in CSF** – neurosyphilis
- **Treponemal serology test only** (TP-PA or FTA ABS) – after screening with nontreponemal testing at a different facility

32

REQUIREMENTS TO REPORT

TESTS OFFERED BY LABORATORIES, cont'd

The number of tests offered by a laboratory ranges, based on available instrumentation, methodology and population requirements.

33

REQUIREMENTS TO REPORT

WHAT TO INCLUDE IN THE REPORT?

- **Algorithm**
  - Final interpretation includes clear statement whether the disease is likely or not.
  - Emphasize results must be interpreted together with clinical symptoms.
  - Results of individual tests are summarized in the interpretive statement.
  - Recommendation regarding retesting is important.
  - Customize interpretive statement for each scenario.
  - Include methodology for automated tests.

34

REQUIREMENTS TO REPORT

EXAMPLE REPORT – REVERSE ALGORITHM

Scenario: Screening test is nonreactive

Reporting individual tests:

Syphilis Interp	Non Reactive
Syphilis Ab CIA	Non Reactive

Interp Statement:

"Consider retesting in 3-12 months if patient remains in high risk category."

35

REQUIREMENTS TO REPORT

EXAMPLE REPORT – REVERSE ALGORITHM

Scenario: Screening test is equivocal, confirmatory tests are nonreactive

Reporting individual tests:

Syphilis Interp	Non Reactive
Syphilis Ab CIA	Equivocal
RPR	Non Reactive
TP-PA	Non Reactive

Interp Statement:

"Because chemiluminiscent screening assay was Equivocal, two confirmatory tests were ordered. Since both RPR and TP-PA are Nonreactive, syphilis is unlikely. Consider repeat serology in 2-3 weeks to rule out early onset of syphilis."

36

REQUIREMENTS TO REPORT

EXAMPLE REPORT – REVERSE ALGORITHM

Scenario: Screening test is reactive, confirmatory RPR is reactive

Reporting individual tests:

Syphilis Interp	Reactive
Syphilis Ab CIA	Reactive
RPR	Reactive
RPR Titer	1:16

Interp Statement:

"Syphilis is likely. Staging of disease requires clinical correlation. Reactive for Treponemal antibodies by CIA screen and non-Treponemal confirmatory test (RPR)."

37

REQUIREMENTS TO REPORT

WHAT TO INCLUDE IN THE REPORT?

- **Individual Tests**
  - Final interpretation includes clear statement regarding the testing outcome, when applicable.
  - Carefully design wording regarding whether syphilis is likely or not (it may be unclear what previous testing was done).
  - Emphasize results must be interpreted together with clinical symptoms.
  - Recommendation regarding retesting is important where applicable.

38

REQUIREMENTS TO REPORT

EXAMPLE REPORT – RPR for confirmation only

Scenario: RPR reactive, previous testing unclear

Reporting individual tests:

Syphilis Interp	Reactive
RPR	Reactive
RPR Titer	1:16

Interp Statement:

"Syphilis is likely. Staging of disease requires clinical correlation. Lab performs and interprets this assay as a follow-up syphilis confirmatory test, presuming other laboratory has ALREADY performed a Treponemal screening test that was reactive. The above interpretation is not valid in the absence of known reactive screening result."

39

REQUIREMENTS TO REPORT

EXAMPLE REPORT – RPR monitor

Scenario: RPR nonreactive, known syphilis patient, monitor treatment

Reporting individual tests:

Syphilis Interp	Non Reactive
RPR	Non Reactive

Interp Statement:

"CDC recommends using a non-Treponemal serologic test (i.e., RPR) to monitor patients diagnosed with syphilis. Non-Treponemal test titers usually decline after treatment and might become non-reactive with time. Interpret results in conjunction with clinical findings."

40

OBJECTIVES

- How to build a syphilis testing algorithm in LIS
  - General guidelines
  - Reflexed testing – correct billing
  - Concrete examples of LIS syphilis test builds

41

SYPHILIS TEST BUILD IN LIS

GENERAL GUIDELINES

- Starts with screening test
- Confirmatory tests are populated by LIS automatically (reflexed testing) **only** when screen is positive or equivocal.
- Each test is reported with a clear interpretation that is transmitted to patient's chart. Customize interpretive statements per scenario, if possible.
- Report all segments at once, after last segment is completed. Do not partially report!

42

SYPHILIS TEST BUILD IN LIS

- **REFLEXED TESTING – Proper billing**
  - Create individual components of the algorithm – can be used as part of multiple tests.
  - Have algorithm built with individual CPT codes for individual components.
  - Do not bundle – reporting and billing would become complicated.

43

SYPHILIS TEST BUILD IN LIS

- **REFLEXED TESTING – Proper billing**
  - Bill clients only for the tests that are reported.
  - Include all segments of individual tests in the build so you can bill for them (example: RPR titers if reflexed).
  - In Test Guide, let clients know that if reflexed testing is performed, another CPT code would be added.

44

LIS REPORTING

- Speaker will provide concrete examples from her laboratory for discussion.

45

REFERENCES

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- CDC: Self-Study STD Modules for Clinicians
- CDC: Sexually Transmitted Disease
- CDC: 2015 Sexually Transmitted Diseases Treatment Guidelines

46

END – THANK YOU

Morality is a veneral disease. Its primary stage is called virtue; its secondary stage, boredom; its tertiary stage, syphilis.

Karl Kraus

47