Advantages of the Reverse Syphilis Screening Algorithm

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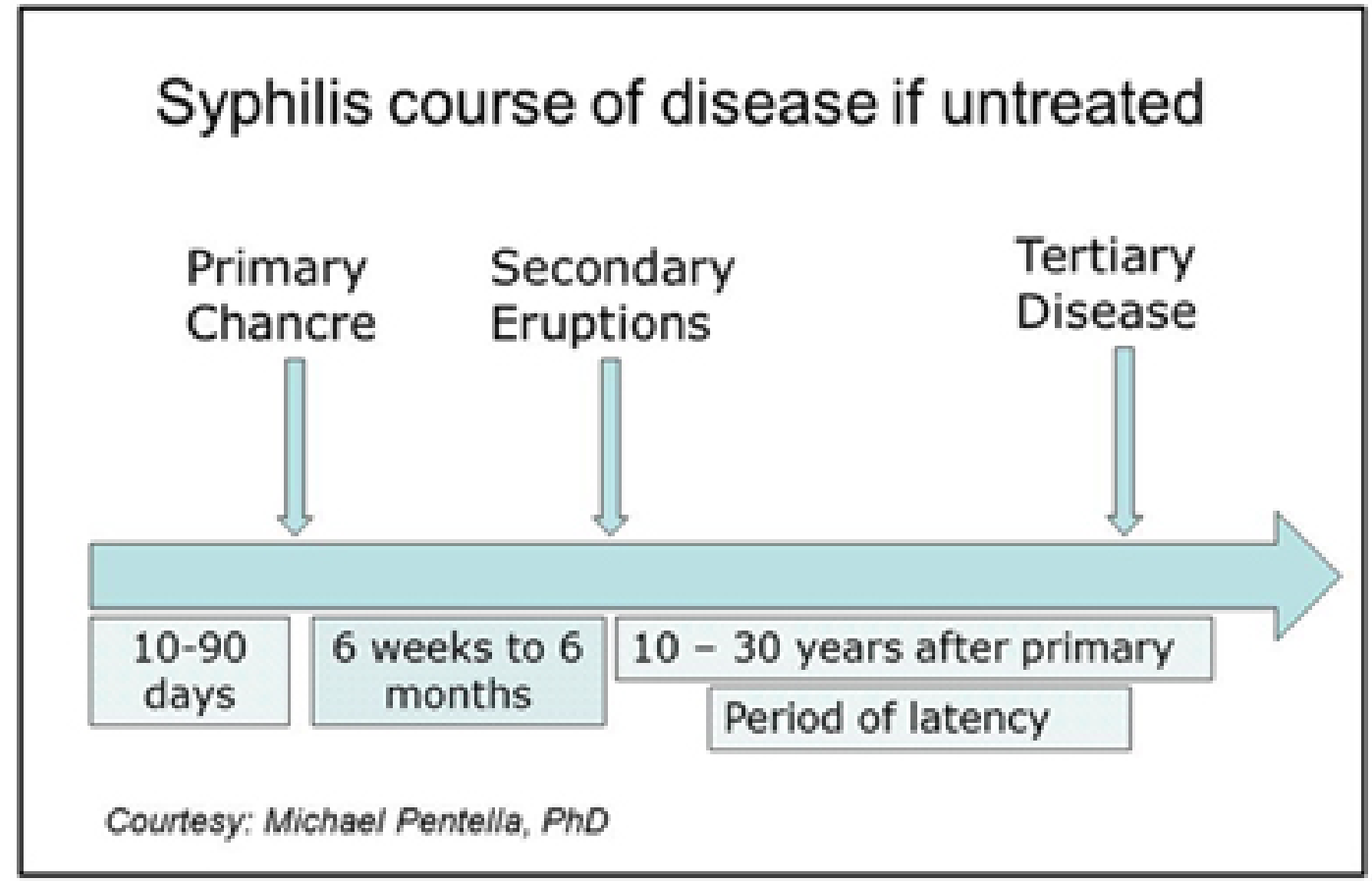
utmb Health

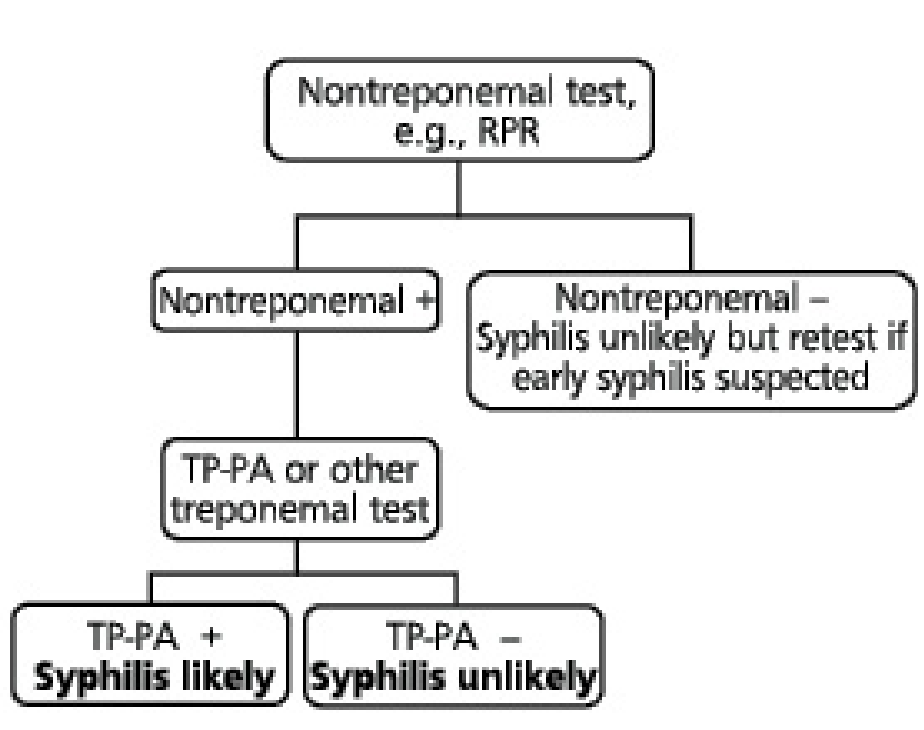
Syphilis is a Complex Disease

Requires a complex testing algorithm



https://labtestsonline.org/understanding/analytes/syphilis/tab/sample/

Traditional Syphilis Testing Algorithm



- False positives
  - Other infections
  - Non-infectious conditions
- False negatives
  - 1° syphilis
  - Prozone

http://www.mlo-online.com/syphilis-evolving-screening-algorithms-and-the-role-of-automated-immunoassays.php

Performance of Nontreponemal Tests

TABLE 2. Sensitivity and specificity of nontreponemal tests

Test	% Sensitivity at given stage of infection				% Specificity (nonsyphilis)
	Primary	Secondary	Latent	Late	
VDRL	78 (74–87)[a]	100	95 (88–100)	71 (37–94)	98 (96–99)
RPR	86 (77–100)	100	98 (95–100)	73	98 (93–99)
USR	80 (72–88)	100	95 (88–100)		99
RST[b]	82 (77–86)	100	95 (88–100)		97
TRUST	85 (77–86)	100	98 (95–100)		99 (98–99)

[a] Range of sensitivity or specificity in CDC studies.
[b] RST, reagin screen test.

Excellent sensitivity for 2° syphilis, reduced sensitivity for other stages, esp. 1° and late

Larsen SA, Steiner BM, Rudolph AH. Clin Microbiol Rev. 1995 Jan;8(1):1-21

Performance of Treponemal Screening Tests

Test	Sensitivity during stage of infection, % (range)				Specificity, % (range)
	Primary	Secondary	Latent	Late	
Nontreponemal tests					
VDRL [14]	78 (74–87)	100	96 (88–100)	71 (37–94)	98 (96–99)
TRUST [14]	85 (77–86)	100	98 (95–100)	NA	99 (98–99)
RPR [14]	86 (77–99)	100	98 (95–100)	73	98 (93–99)
Early treponemal tests					
MHA-TP [15]	76 (69–90)	100	97 (97–100)	94	99 (98–100)
TPPA [16]	88 (86–100)	100	100	NA	96 (95–100)
TPHA [17]	86	100	100	99	96
FTA-ABS [14]	84 (70–100)	100	100	96	97 (94–100)
Enzyme immunoassays					
IgG-ELISA [18]	100	100	100	NA	100
IgM-EIA [19]	93	85	64	NA	NA
ICE [20]	77	100	100	100	99
Immunochemiluminescence assays					
CLIA [21]	98	100	100	100	99

NOTE. CLIA, chemiluminescence assay; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay; FTA-ABS, fluorescent treponemal antibody absorption assay; ICE, immune-capture EIA; MHA-TP, microhemagglutination assay for *Treponema pallidum;* NA, not available; TPHA, *T. pallidum* hemagglutination assay; TPPA, *T. pallidum* particle agglutination; TRUST, toluidine red unheated serum test.

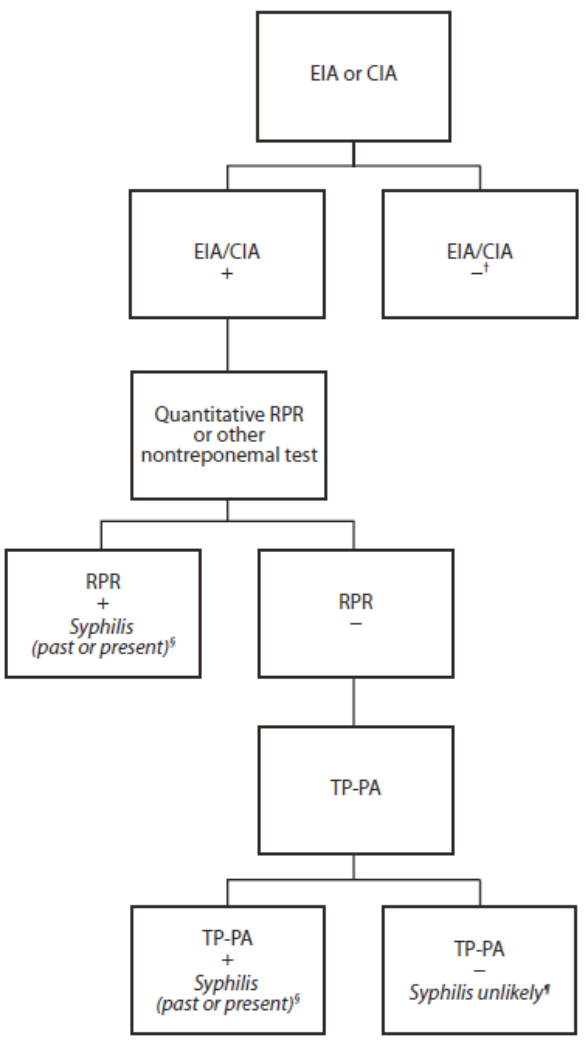
Sena AC, White BL, Sparling PF. Clin Infect Dis. 2010 51:700-8

Performance of Treponemal Screening Tests

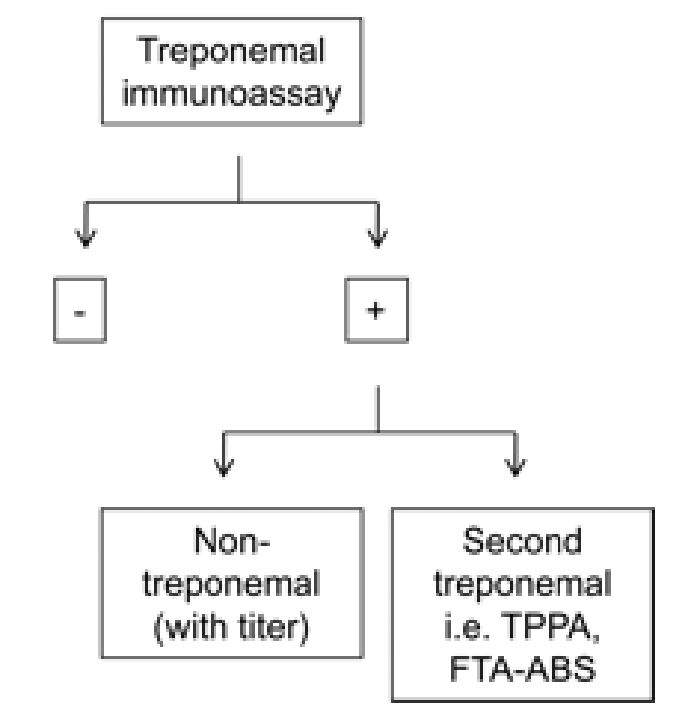
- More sensitive than nontreponemal tests during primary, latent and late syphilis
  - Important criterion of screening tests- sensitivity!
- However, like any highly sensitive screening assay, false positives occur. The proportion of false positives among all positives increases as disease prevalence decreases.

Reverse Syphilis Algorithm

CDC



UTMB



https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6005a1.htm

Loeffelholz MJ, Binnicker MJ. J Clin Microbiol. 2012 Jan;50(1):2-6

Syphilis Testing at UTMB

- About 3900 screening tests per month

Population	Proportion of test volume	Screen reactive rate
OB/GYN	~63% (of these, about 11% are high-risk L&D)	1.8%
Incarcerated	~28% (85% male)	7.5%
Misc. inpatient, clinics	~9%	5%

- Overall, about 150 supplemental tests per month
- In 2009 we switched from RPR to treponemal IgG as our screening test

Important Differences

Nontreponemal tests

- Detect reagin (anti-lipid) antibodies, using cardiolipin, lecithin and cholesterol antigens
- Primarily detect Abs formed as a result of damage to tissue
- Usually reactive only during active, untreated syphilis

Treponemal tests

- Detect anti-*Treponema pallidum* antibodies
- Cannot differentiate between active and past treated infection

Performance of Reverse Algorithm Sequence

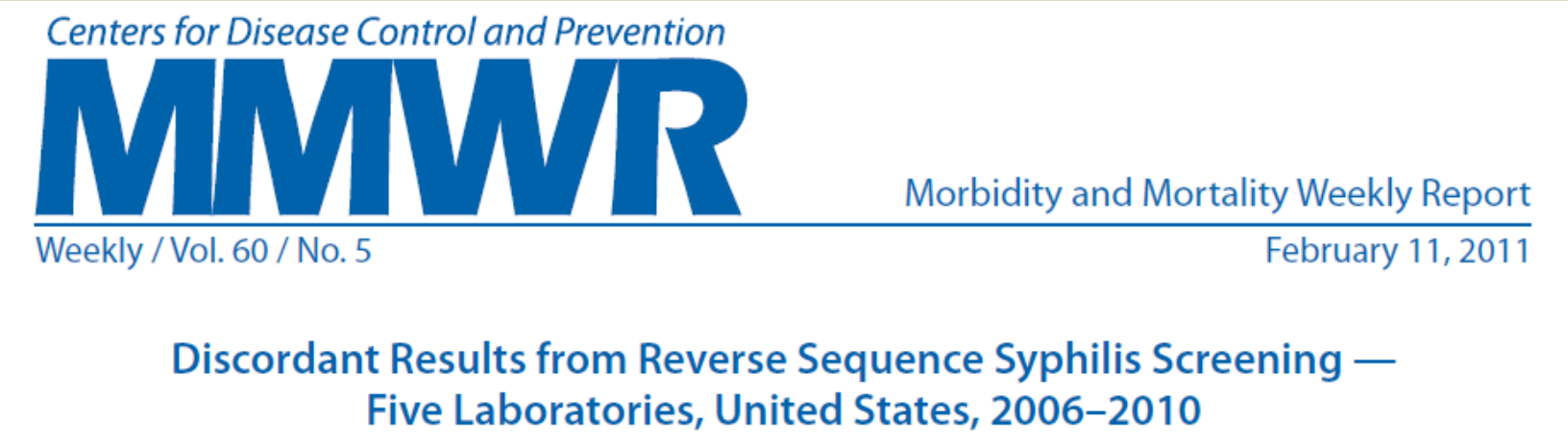
- When specimens with reactive treponemal screening results are reflexed to supplemental tests, specificity is excellent
- Other advantages- automation

Endorsement of Reverse Algorithm

- Canada (Public Health Laboratory Network)
  - "Both traditional and reverse sequence algorithms are acceptable for the serological diagnosis of syphilis infection."
- Europe (International Union against Sexually Transmitted Infections (IUSTI))
  - "A treponemal antigen test EIA or TPPA…is recommended as a single screening test…The RPR/VDRL is not recommended as a primary screening test."
- United Kingdom (Public Health Laboratory System)
  - "Screening with a non-treponemal test alone is not recommended because of the potential for false negative results." "A treponemal EIA alone… is appropriate for screening…"

Levett PN et al. 2015. Can J Infect Dis Med Microbiol
French P et al. 2009. Int J STD & AIDS
Egglestone SI et al. 2000. Comm Dis Public Health

However, U.S. is Hesitant



- High proportion (57%) of specimens with reactive EIA/CIA screening test had a nonreactive RPR test
  - UTMB: 46% of IgG reactive are RPR nonreactive. However, of these "discordant" specimens, 2/3 are TPPA reactive (strong evidence for past treated infection)
- CDC: "…continues to recommend that nontreponemal tests be used to screen for syphilis…However, if reverse sequence screening is used…specimen with reactive EIA/CIA results be tested reflexively with a quantitative nontreponemal test…"

Challenges of Reverse Algorithm

- Clinician buy-in
  - Numerous studies show treponemal EIA tests have equivalent or greater sensitivity than nontreponemal tests
  - Interpretation of IgG R/RPR NR results in patients without history of treatment can be difficult
- Ongoing education
- Reporting of results (result interpretation)
  - Supplemental test(s) should be performed quickly on initially reactive specimens. All test results reported together, and interpretation attached.

Interpretation of Reverse Algorithm Results—UTMB

	Syphilis IgG	RPR titer	TPPA	Interpretation
96%	Nonreactive	*(not performed)*	*(not performed)*	No serologic evidence of T. pallidum infection. Cannot exclude incubating or early syphilis. Submit second sample in 2-4 weeks if clinically indicated.
0.6%	Reactive/ Equivocal	Nonreactive	Nonreactive	**UNLIKELY:** Based on IgG, RPR, and TPPA results, previous T. pallidum infection unlikely.
1.1%	Reactive/ Equivocal	Nonreactive	Reactive	**PAST:** Based on (all) results, suggests past or treated syphilis; however, clinical correlation is needed.
2%	Reactive/ Equivocal	Reactive	Reactive	**ACTIVE:** Based on (all) results, probable active T. pallidum infection.
<0.1%	Reactive/ Equivocal	Reactive	Nonreactive	**POSSIBLE*:** Based on (all) results, possible infection or biological false positive. Submit second sample in 2-4 weeks if clinically indicated.

* CDC considers this to be confirmed, active syphilis

Conclusions

- Reverse syphilis testing algorithm is more sensitive than traditional algorithm
  - Supplemental testing (RPR titer) distinguishes active from past treated infections
- Treponemal screening by immunoassay can be automated
- Implementation hindered by lack of understanding
  - Laboratory needs to communicate benefits (sensitivity)
  - Laboratory needs to provide clear interpretation of results