

LABletter

PATHOLOGY & LABORATORY MEDICINE NEWSLETTER

FEATURE ARTICLE HIGHLIGHTS>>

*New ELISA Screening for Syphilis
...Flipping the Treponemal Paradigm*

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2. Treponemal & Non-Treponemal Antibodies
3. New Approach to Syphilis Testing with Treponemal ELISA...
 - A. Detects IgM anti-*T. pallidum* in addition to IgG antibodies
 - B. Reduction of both false negative and false positive
 - Better Sensitivity
 - Increased Specificity
 - Increased Efficiency
4. Monitoring Treatment with RPR

Laboratory Update:

New Syphilis Assays
Complexed PSA
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Q&A:

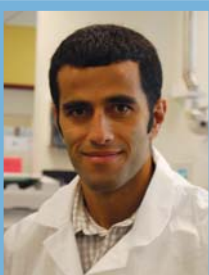
Respiratory Viral Testing

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New ELISA Screening for Syphilis *...Flipping the Treponemal Paradigm*

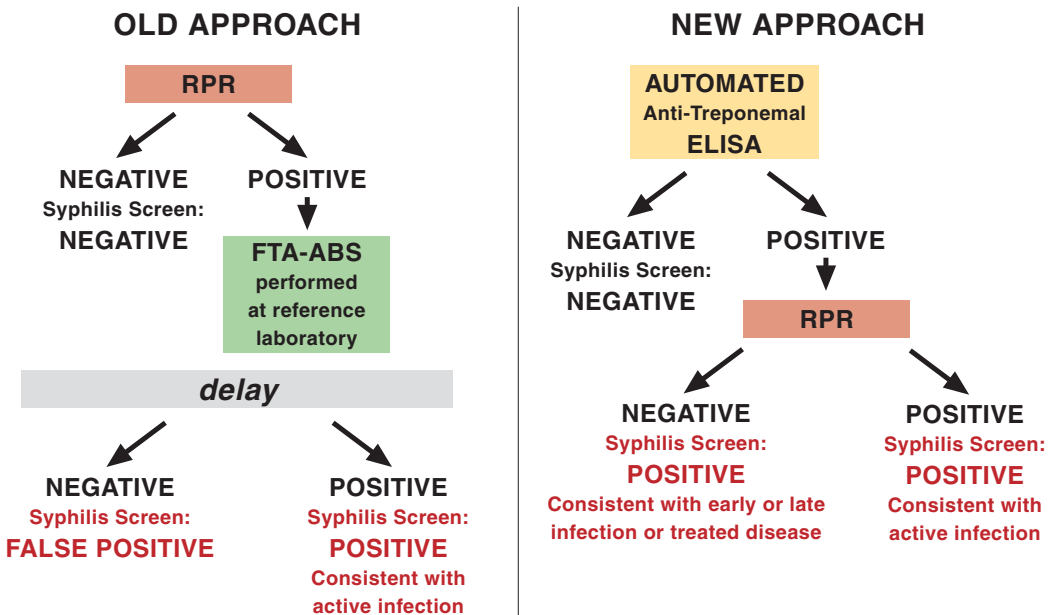
The incidence of syphilis declined after the introduction of penicillin 60 years ago but it is still a significant health problem, especially in developing countries. Although in the U.S. at the end of the twentieth century the incidence was probably the lowest ever recorded. The disease is still endemic and has recently surged in some urban areas.

Because the causative agent *Treponema pallidum* cannot be grown using conventional techniques, serology is the primary method for laboratory diagnosis of syphilis. Syphilis

antibodies (and serological tests for syphilis) are divided into two types: non-treponemal and treponemal. In response to infection with *T. pallidum*, the immune system produces both.

Traditional screening for syphilis infection involves using a "non-treponemal" test, followed by a confirmatory "treponemal" test. At Stanford, we are planning to reverse this approach by implementing an automated treponemal ELISA for screening, followed by the RPR test to determine if the patient has active disease (see Fig. 1).

The Old and the New Approach to Syphilis Testing (Fig. 1)



Screening with a non-treponemal test (old approach) may miss very early primary infection and late or latent infection. But the major problem with the old approach is that positive results require FTA-ABS confirmation adding significant delay to the turn around time.

“New Approach to Syphilis Testing screening with a sensitive and specific treponemal assay...Reduction in both false-negative and false-positive results...Differentiation of prior treated infection and active infection”

Non-Treponemal Antibodies

The non-treponemal antibodies are directed against phospholipids released from the tissue invaded by the bacteria. The traditional serologic approach to the diagnosis of syphilis involves identifying these antibodies using flocculation of the phospholipid cardiolipin in a mixture of cholesterol and lecithin. The original assay (*Venereal Disease Research Laboratory, or VDRL, test*) required that this mixture be made up each day and that the tests be examined under a microscope.

Today, most laboratories use the *Rapid Plasma Reagin (RPR)* test in which the lipid suspension is stabilized and charcoal particles are added to allow the flocculation to be observed visually without a microscope. Although this is easier to perform than the VDRL test, it is still relatively labor-intensive, subjective, and prone to false-negative results (if the level of antibody is very high and the flocculation is inhibited in a phenomenon called "pro-zone").

But the major problem with using the RPR as a screen is that anti-cardiolipin antibodies are not specific for syphilis; up to 50% of positive RPR results may be false-positives (especially in low-risk populations). Therefore, positive screening results using RPR required confirmation using a treponemal-specific antibody assay.

The traditional confirmatory assay employed indirect immunofluorescence, using actual organisms (fluorescent treponemal antibody-absorbed, or FTA-ABS test). More recently, most laboratories moved to an agglutination assay in which

treponemal antigens are coated on either red blood cells (MHA-Tp) or colored gelatin particles (TPPA). At Stanford, we had been forwarding RPR-positive specimens to the Santa Clara County Public Health Laboratory for TPPA testing, but because of long turnaround times, we recently began using a reference laboratory that performs FTA-ABS.

When this traditional approach was first introduced, labor costs were relatively low and the costs of the treponemal assays such as FTA-ABS were relatively high. With the introduction of automated treponemal-specific enzyme-linked immunoassays ("ELISAs"), the reverse is now the case.

Screening with Treponemal ELISA

The new approach to syphilis screening uses a new version of the Phoenix Bio-Tech Corporation's Trep-Chek™ immunoassay, called Trep-SURE™. This assay detects IgM anti-Treponemal in addition to IgG antibodies (see Fig. 2).

Although you may still order qualitative RPR as a screening test for syphilis, we hope that you will consider switching to the new approach for the following reasons. The new approach has better sensitivity; RPR is a difficult test to interpret when low levels of non-treponemal antibody are present and the test may be negative during early or late infection. The new approach has increased specificity; most positive RPR results are false-positives, especially when screening low-risk populations. Finally, the new approach is more efficient. The results of positive anti-treponemal antibody screens can be reported at the same time as the other results; positive RPR specimens must be sent for FTA-ABS confirmation. Also, because the RPR is a manual, labor-intensive test while the anti-treponemal immunoassay is completely automated, the overall cost of the two approaches is comparable.

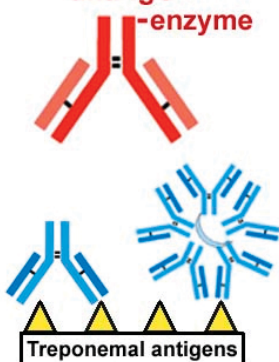
RPR for Monitoring of Treatment

Note that we now have separate order codes for qualitative (screen) and quantitative (follow-up) RPR testing (see new test code in New Test Update on following page). Physicians who wish to monitor their patients' response to antibiotic therapy using the RPR titer should order the new quantitative RPR test. A two-fold decrease in titer indicates an appropriate response in patients with primary or secondary infection. Note that FTA-ABS confirmation will no longer be automatically performed for quantitative RPR testing. These changes to Syphilis Screening take effect in January, 2008.

Please see the Laboratory Update on the next page for more information about these new order codes.

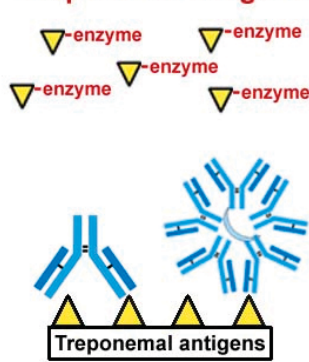
Treponemal Assays by ELISA (Fig. 2)

Trep-Chek™ assay
anti-IgG
-enzyme



The current immunoassay for anti-treponemal antibodies, Trep-Chek™, uses an enzyme-labeled anti-IgG antibody to detect immunoglobulins bound to the treponemal antigens on the solid phase; the assay will not detect IgM antibodies.

Trep-SURE™ assay
Treponemal antigens



Stanford is using Trep-SURE™, a new more sensitive version, which uses enzyme-labeled antigens to detect both IgG and IgM antibodies.

Laboratory Update:

Syphilis Changes and eGFR Update

We recommend that Syphilis Treponemal Screen (STSS) be ordered when screening a patient for infection with *Treponema pallidum*. Positive results using anti-Treponemal ELISA do not need to be sent for confirmatory testing.

Syphilis Treponemal Screen

Order Code: STSS

Synonyms:	ELISA Syphilis Screen
Specimen Type:	Serum
Container Type:	SST Gold-top tube
Required Volume:	1 mL
Methodology:	Anti-treponemal immunoassay (ELISA) Includes RPR titer if positive
Reported:	24 hours / next day
Reference Range:	Negative
CPT Codes:	86592

Rapid Plasma Reagin, qualitative (RPRQL) may still be used as a screen for syphilis but positive results will be delayed due to the need for FTA-ABS confirmation. Rapid Plasma Reagin, quantitative with titer (RPRQT) should be ordered as follow-up testing to monitor treatment of patients with known infection. These test results will not be confirmed by treponemal-specific assays.

Rapid Plasma Reagin

Qualitative Order Code: RPRQL

Quantitative Order Code: RPRQT

Synonyms:	RPR Syphilis Screen (RPRQL) RPR titer (RPRQT)
Specimen Type:	Serum
Container Type:	SST Gold-top gel tube
Required Volume:	1 mL
Methodology:	Lipid flocculation RPRQL includes FTA-ABS confirmation if positive RPRQT does not include FTA-ABS confirmation
Reported:	RPRQL: 24 hours / next day if negative; 2-4 days if positive RPRQT: 24 hours / next day
Reference Range:	Negative
CPT Codes:	Qualitative - 86592, Quantitative - 86593

Update regarding changes to PSA testing (See original article in September-October issue of the LabLetter)

To help physicians cope with the conversion from free to complexed PSA, we have added calculated free PSA to the "Total & Complexed PSA" battery. The interpretative comment will still appear as part of the % complexed PSA, but the physicians will be able to see what the free PSA would have been. This calculated free PSA correlated beautifully with the Hybritech free PSA assay in our validation.

Complexed PSA is the "tumor" PSA, now measured (and expressed) directly. Also, because we are lowering the "cut-off" for total PSA to 2.5 ng/ml, the complexed assay may help to interpret the risk of cancer better in the lower total PSA ranges (<4) than the free PSA does.

- If the free PSA was <10%, this indicated cancer; if the complexed PSA is >90%, this indicates cancer.
- If the free PSA was >20%, this made cancer less likely; if the complexed PSA is <80%, this makes cancer less likely.
- If the free PSA was between 10-20%, the test didn't help very much; if the complexed PSA is between 80-90%, the test doesn't help very much.

If you have any questions, please call Customer Service at 1-877-717-3733 and request to speak to Dr. James Faix.

eGFR Update

Our recent implementation of eGFR was very well received, however a number of physicians have asked why the eGFR calculation does not appear for all patients. As described in our July/August LabLetter, the eGFR is not reported in all test panels containing serum creatinine

Estimated GFR Calculation

Order Code: EGFR

Synonyms:	eGFR
Specimen Type:	Plasma / Serum
Container Type:	Preferred: Mint-top gel tube
Required Volume:	1 mL
Methodology:	Colorimetric (Creatinine assay with eGFR calculation)
Reported:	24 hours / next day
Reference Range:	>60 mL / min. / m2
CPT Codes:	82540

under specific circumstances (eGFR>60 ml/min/m2; Creatinine >2.0 ml/dl; hospitalized patients and for patients < 18 years of age).

By popular request, eGFR is now available under a separate test code. The result will appear with comment noting the chronic kidney disease (CKD) stage based on National Kidney Foundation (NKF) guidelines, www.kidney.org.

eGFR	CKD Stage	NKF Recommendation
>60	1 or 2	No or mild decrease in GFR Diagnosis & treatment Slow progression CVD risk reduction
30-59	3	Moderate decrease in GFR Treat complications
15-29	4	Severe decrease in GFR Prepare for renal transplantation
<15	5	Kidney failure Renal transplantation

Q&A: Submit Q&A topics of interest to: labmarketing@stanfordmed.org

An opportunity for the Bay Area Medical Community to request specific Q&A topics to our medical directors that are relevant to patients' clinical needs.



Bruce Patterson, M.D.

Respiratory Viral Testing

Q&A with Bruce Patterson, M.D., Director of Virology at Stanford University Medical Center Clinical Laboratories

Q1: What is the role of laboratory testing for influenza A or B during peak outbreaks?

A1: The role of the laboratory in the peak respiratory virus season, which usually runs from November to April each year, is to provide the most rapid and accurate diagnostic information as possible. This information is critical for possible prophylaxis, treatment, and prevention of transmission.

Q2: Which laboratory test is the “gold standard” for influenza, viral culture or PCR?

A2: There is considerable debate about what test is the “gold standard” for respiratory viruses. Though amplification techniques such as PCR, real-time PCR, and gene chips (solid or liquid) seem to be the logical choice as a gold standard, FDA and others consider viral culture to still be the gold standard. At Stanford, we use amplification techniques such as PCR as our “gold standard” in evaluating new technologies.

Q3: Are rapid antigen tests for influenza A or B antigens sensitive enough?

A3: Rapid antigen tests for Influenza A, Influenza B, and RSV are used extensively around the world. They are generally of the class called lateral flow devices and have high analytical sensitivity. During periods of high prevalence, these rapid antigen tests have clinical sensitivity and specificity ranging from 80% to 95%.

However, of critical significance, during low prevalence months these rapid tests can have clinical sensitivities and specificities around 50% making it extremely difficult to reliably interpret results.

Consequently we rely on the direct viral examination (DFA) using monoclonal antibodies to diagnose the rare case of influenza or RSV in low prevalence months. The DFA test has relatively high clinical sensitivity and specificity even in low prevalence times. The DFA test is offered twice a day at Stanford with 9:00 and 4:00 cut-off times for specimen processing.

Q4: When should physicians also screen for RSV?

A4: The peak season for RSV is generally between November and April, though we have had several diagnosed cases in the summer of 2007. We recommend using the rapid tests to screen between October 15th and April 15th and the Respiratory Virus Panel by DFA during the low prevalence months.

Q5: What is the specimen of choice for Respiratory Virus testing?

A5: At Stanford we utilize the Copan flocced nasopharyngeal collection device for best results. Flocced nasopharyngeal swabs

have demonstrated the same clinical sensitivity and specificity to that of nasopharyngeal aspirates and have been validated by Stanford Department of Virology transported at room temperature in Viral Transport Media (VTM). Stanford provides a Respiratory Virus Nasopharyngeal Specimen Collection Kit containing 2 Copan nasopharyngeal flocced swabs with a VTM tube.

Q6: When is the best time to collect a specimen for Respiratory Virus testing?

A6: Viral shedding is higher in young children during the first days of disease. Specimens should be collected within 4 days of disease onset. Collect 1-2 nasopharyngeal specimens and place in VTM tube. Transport to laboratory as soon as possible at room temperature or refrigerated.

Q7: Do the current tests detect avian flu?

A7: Both our current rapid influenza test and our Respiratory Virus DFA panel will detect that influenza A is present though it will not distinguish between seasonal Influenza A and Influenza A (avian H5N1). Stanford is taking part in the FDA trials for a rapid device that will distinguish between seasonal serotypes and the avian H5N1 strain. This test takes 15 minutes to perform and has 400 times the sensitivity of routine lateral flow devices for the detection of influenza. This test will be performed at Stanford in January as part of the initial clinical trials.

Weekly updates regarding flu activity are available from the CDC and may be found at www.cdc.gov/flu/weekly/fluactivity.htm



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