REX

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UPDATES AND INFORMATION FROM REX PATHOLOGY LABORATORY

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Change in Syphilis Reference Testing

The Mayo Clinic no longer offers the fluorescent treponemal antibody absorption (FTA-ABS) test. It has been replaced with the syphilis antibody, IgG. If the IgG is positive then Mayo reflexively performs a rapid plasma reagin (RPR) card test. An IgM syphilis antibody test is also available. The new tests are enzyme immunoassays (EIA).

Background

Syphilis is caused by the spirochete, *Treponema pallidum*. The organism cannot be successfully cultured and serology is the principle method for diagnosis. Tests fit into two major categories, treponemal tests and nontreponemal tests. The traditional work-up of a suspected case of syphilis has been to start with one of the nontreponemal screening tests, which can detect antibodies that react with lipoidal particles containing cardiolipin, a phospholipid released by damaged host cells. Positive results were then titered and confirmed with a treponemal test, which use *T. pallidum* as the antigen to detect antibodies formed against the organism.

This has been the traditional approach because the nontreponemal tests are inexpensive and relatively simple to perform while the treponemal tests are costly, more complex to perform, and cannot be used to monitor reinfection or the effect of treatment since they usually remain positive for life. Table 1 compares the most commonly used syphilis tests.

The newer EIA tests have reported sensitivities between 97-100% and specificities of 98-100%. The EIA test at Mayo (TREP-CHECK Antitreponemal EIA, Phoenix Bio-Tech Corp., Ontario, Canada) spectrophotometrically measures color change to give a result as opposed to the nontreponemal tests, which rely on subjective determination of visual flocculation.

The many different assays do have false positive results. Autoimmune diseases can lead to antibodies that will affect many of the nontreponemal tests including the RPR. Lyme disease is an example of a disease more likely to cross-react with a treponemal test.

With the introduction of EIAs for the diagnosis of syphilis, many clinicians have reversed the traditional order of testing. A positive EIA is followed by a nontreponemal test (RPR at the Mayo Clinic). As rates of syphilis continue to decline, most initial positive results in low risk populations will be false positives and clinical correlation will be necessary so that the appropriate group of patients is tested.

An order for an FTA-ABS will be sent to Mayo for a syphilis IgG since most tests are ordered for secondary or latent syphilis. Ocular manifestations are late findings and are sufficiently screened for by an IgG test. If acute/primary syphilis is suspected then the lab should be contacted and a syphilis IgG and IgM will be ordered. Table 2 highlights interpretation of the syphilis IgG and IgM panel from the Mayo Clinic.

Table 1 Sensitivity and specificity of serologic tests for syphilis (Adapted from table 3, p 960, Manual of Clinical Microbiology 2003).

	Sensitivity(%)				Specificity(%)
Test	Primary	Secondary	Latent	Late	
Nontreponemal					
VDRL	78	100	95	71	98
RPR card	86	100	98	73	98
USR	80	100	95		99
TRUST	85	100	98		99
Treponemal tests					
FTA-ABS	84	100	100	96	97
FTA-ABS DS	80	100	100		98
TP-PA	88	100	100		96

USR = unheated serum reagin
TRUST = toluidine red unheated serum test
DS = double staining
TP-PA = *Treponema pallidum* particle agglutination which has replaced the microagglutination assay for antibodies to *Treponema pallidum* (MHA-TP).

Table 2: Interpretive results for the syphilis panel (Adapted from Mayo Reference Services New Test Announcement November 2004)

Result	IgM	IgG	RPR*
Active or recently treated syphilis	+	+	+
Active or recently treated syphilis	+	+	-
Acute (primary) syphilis	+	-	+
Acute (primary) syphilis	+	-	-
Active or recently treated syphilis	-	+	+
Past, successfully treated, or latent syphilis **		-	+
No evidence of active syphilis***	-	-	Not performed

* The RPR is a reflex test for a positive syphilis IgG or IgM. RPR may be useful for determining the current disease status and response to therapy. RPR titers should decrease with successful treatment over time.
** Infants <6 months with IgG or positive RPR, without IgM have probable maternal antibody.
***Severely immunocompromised patients with active syphilis can test negative. Very early primary syphilis and successfully treated (>10 years ago) can also have negative tests. Response to treatment may be indicated by a decrease in RPR titers or the reversion of a positive to negative IgM result.
Positive and equivocal IgM results occurred in patients with the following conditions:
Cardiolipin IgM antibodies (1 of 10 positive)
Anti-ANA, anti-DNA antibodies (3 of 10 positive, 2 of 10 equivocal)
EBV VCA IgM antibodies (2 of 5 positive, 1 of 5 equivocal)
Borrelia IgM antibodies (1 of 10 positive, 1 of 10 equivocal)
CMV IgG antibodies (2 of 5 positive)
Parvovirus B19 IgM antibodies (1 of 5 positive)
Rheumatoid factor IgM (0 of 10 positive)

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Testing at Rex

Rex will continue to offer an in-house RPR card test Monday through Friday. If there is a positive result the specimen will be titered and reported. All positive specimens will be forwarded to the North Carolina State Department of Health for a TP-PA. If the TP-PA is reactive, state law requires completion of a Department of Health Confidential Report Form.

Collection and Ordering Information

- The RPR at Rex or the Syphilis IgG and/or IgM at Mayo both require a red top tube of blood.
- If an FTA/ABS is ordered a syphilis IgG will be ordered at the Mayo Clinic (Mayo test code 81814).
- A syphilis IgM can be ordered independently (Mayo test code 84419).
- If the full panel is desired (because primary or congenital syphilis is a consideration) then request syphilis IgG and IgM (Mayo test code 84425).

If there are any questions contact the reference desk at (919) 784-4117 or Dr. Smith at (919) 784-3056.

Vincent C. Smith M.D.

References:
1. *Murray, PR. Manual of Clinical Microbiology. American Society of Microbiology Press 2004, p955-966.*
2. *Mayo Reference Services. New Test Announcements, November 2004.*

Colposcopic Endocervical Sampling

Traditionally colposcopic evaluation has included sampling of the endocervical canal using endocervical curettage (ECC). This has been recommended even when the colposcopic examination has clearly delineated the lesion and when the squamocolumnar junction is well visualized. Unfortunately this process is fraught with difficulties related to sensitivity, specificity and specimen adequacy. For example, one study using cone biopsies performed immediately after ECC's in patients with High Grade lesions demonstrated that the ECC failed to detect endocervical canal involvement in fifty percent of cases.[1] Conversely false positive rates for ECC have been estimated at 9-15%[1].

Most of the false positive cases have been detected in women in whom the ECC was performed despite adequate colposcopy and good visualization of a normal appearing endocervical canal. False positive cases are also increased when the ECC is performed after the exocervical biopsies since this increases the risk of inadvertent contamination of the ECC specimen. In order to reduce false positive results, the ASCCP recommends that ECC not be performed when colposcopy is satisfactory and the endocervical canal is well visualized. However, in women who have had prior cervical therapy such as cryotherapy, laser, LEEP, loop or cold-knife conization there may be skip lesions within the endocervical canal and thus ECC is recommended even when the colposcopy is otherwise satisfactory. Any women with glandular abnormalities on the Pap test also require ECC evaluation. Since most false positive ECC's result in a "single detached fragment" of abnormal epithelium in the specimen it is recommended that ECC specimens be obtained using colposcopic visualization with documentation of any

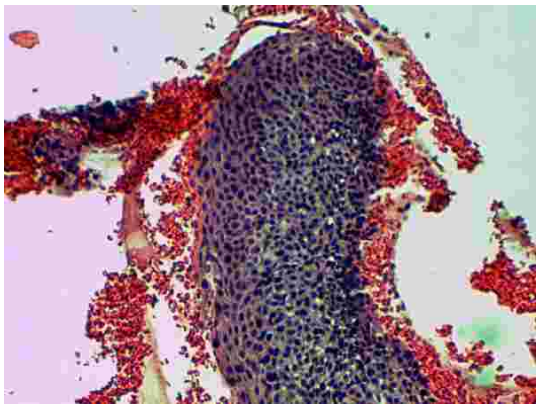

Image 1: Detached fragment of high grade dysplasia in endocerical curetting.

inadvertent scraping of the exocervix. It is also recommended that ECC samples be collected prior to any exocervical samples. If a positive ECC occurs in association with an inadequate colposcopy, a poorly visualized endocervical canal, or when multiple fragments of dysplastic epithelium are present then it must be assumed that it represents a true positive. If, however, a "single detached fragment" of abnormal epithelium is present in an ECC performed with adequate colposcopy and good visualization of a normal appearing endocervical canal then the sample may represent a false positive. In these cases the ASCCP recommendation is to repeat the ECC in 4 to 6 weeks and if it is negative then document all findings in the chart and continue to follow the patient as if the ECC were a true negative.[1]

On the other hand, false negative ECC are the result of inadequate sampling. Several studies have documented that the sensititivity for ECC is proportional to the amount of tissue obtained for examination.[1,2] Therefore a negative ECC in the face of a limited or sparse or scanty specimen should not be considered to be a true negative. In these situations, or when the ECC is frankly inadequate, the ASCCP recommends repeating the ECC in 4- 6 weeks.[1].

There has been recent interest in using a sleeved cytobrush rather than an endocervical curette for obtaining the

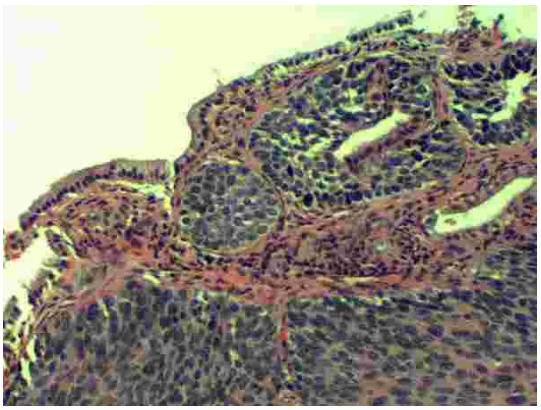

Image 2: High grade dysplasia involving endocervical gland. Definitive evidence of endocervical canal involvment by dysplasia.

endocervical sample. A recent study has demonstrated that the sleeved cytobrush achieves sensitivity and specificity similar to that seen with the endocervical curette and has a much higher specimen adequacy rate.[2] The sleeve that guides the cytobrush also protects against false positive specimens by protecting the brush from contamination from proximal lesions on the exocervix. If using a sleeved cyobrush specimen you may submit it to the laboratory as either a cytology or surgical pathology specimen. It is preferred that you submit these specimens for surgical pathology evaluation when accompanied by cervical biopsies and that you do so by simply detaching the head of the endocervix brush and placing it in a vial of formalin labeled as endocervical brushing. If you do decide to submit the sample as a cytology specimen then simply detach the head of the device and place it into a SurePath™ Pap Test collection vial. *It is critical that you note on the requisition that the specimen is not a Pap Test but is a specimen to evaluate the endocervical canal for dysplasia.* Of note is that the ASCCP currently lists either the traditional endocervical curettage or the sleeved endocervical brush as alternatives for sampling of the endocervical canal.[1]

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References
1. *Cox JT. ASCCP Practice Guidelines: Endocervical Curettage. J Lower Genital Tract Dis 1997;1:251-6.*
2. *Boardman LA, et al. A Randomized Trial of the Sleeved Cytobrush and the Endocervical Curette. Obstet Gynecol 2003;101:426-30.*

Brief Case Review
Abnormal Bleeding following a broken hip

Clinical history:

A 51-year-old woman is admitted to the hospital after falling down a flight of stairs and sustaining a hip fracture. She developed a large hematoma over the hip following the traumatic fracture. On further questioning she informs her physician of prolonged oozing after dental procedures and a positive family history of bleeding. She is on low dose aspirin to control cardiovascular risk factors. The surgeon orders a CBC, PT and aPTT prior to the surgical repair of the hip and anticipates a drop in the hemoglobin level because of the appearance of the hip.

Lab data:

Laboratory results are as follows: PT = 12 sec, (normal 12 – 15 sec), aPTT = 48 sec (24 – 36 sec), hemoglobin of 9.5 g/dl and a normal platelet count. To evaluate the prolonged aPTT, the attending physician orders a mixing study and assays of factors VIII and IX plasma levels. The aPTT corrects to normal with the addition of normal plasma. The factor VIII and IX levels are normal (92% and 112% respectively). You are consulted because the orthopedic surgeon wants to know the nature of the bleeding abnormality and how to prevent surgical bleeding during the operative repair.

Hint: Her Rabbi is visiting when you arrive for the consultation.

Questions:

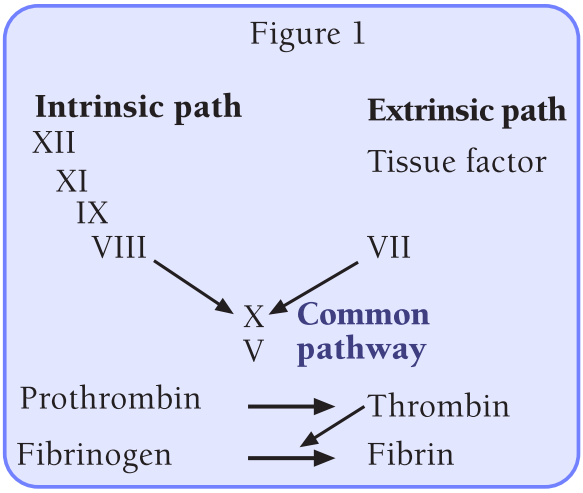
1. What is the likely diagnosis?
2. What family history question could be asked to confirm your suspicion about the likely diagnosis?
3. What additional laboratory test(s) would you order?
4. How should this patient be treated to prevent bleeding from orthopedic surgery?
5. How should this patient be treated for future dental surgery?

Case Discussion (Answers):

Introduction:
The classic cascade of coagulation factors to form a hemostatic plug is illustrated in figure 1. Although the cascade is an over simplification of hemostasis, it is helpful conceptually to list the coagulation factors measured by the different lab tests. The aPTT (activated partial thromboplastin time) measures the activity of the factors in the intrinsic pathway and final common pathway. A prothrombin time (PT) measures the extrinsic pathway and final common pathway. The thrombin clotting time (TCT) measures the conversion of fibrinogen to fibrin. The mixing study is used to discriminate between factor deficiencies and plasma inhibitors. A mixing study is a reasonable starting point in the evaluation of a prolonged aPTT because it helps separate a factor deficiency from an inhibitor. Inhibitors cause the aPTT to remain prolonged after mixing normal plasma with the patient's plasma. The aPTT is corrected with the addition of normal plasma if there is a factor deficiency. Some inhibitors are uncovered only after mixing studies are incubated for 30 minutes at 37 degrees centigrade.

1. This patient has an isolated prolonged aPTT value associated with bleeding. These findings strongly suggest a deficiency or inhibitor to factors VIII, IX or XI. The first two factors have been assayed and the values are normal. Factor XII (Hageman factor) is part of the intrinsic pathway and is not associated with bleeding but does cause a prolonged aPTT. The mixing study corrects the aPTT to within the normal range at room temperature and after incubation. This study rules out an inhibitor and a Factor XI deficiency becomes a likely cause.

Figure 1



2. Although factor XI deficiency occurs in non-Jews, it is more common in Jews. A factor XI deficiency is suspected by the family history and ethnicity. Her pre-op factor XI level is 6% (80 – 100%). Factor XI deficiency (hemophilia C) has a gene frequency of 5 to 10% in Ashkenazi Jews. Those individuals who have a bleeding disorder are

almost always homozygous for the autosomal recessive gene responsible for the deficiency. It should be understood that aPTT laboratory reagents vary in their ability to detect mild factor XI deficiency and many patients with this deficiency may have normal or only minimally abnormal aPTT values. *If clinical suspicion is high, a factor assay is warranted even if the aPTT is normal.*

3. Sterile factor XI concentrates have been used in Israel but have significant risk of thrombosis. Fresh frozen plasma is appropriate in mild disease and should be administered to cover major surgery. The half-life of factor XI is 2 or 3 days and is stable for the same period. The minimum hemostatic level of factor XI is a blood level of 10 to 20%. One unit of fresh frozen plasma has about 200 to 250 units of factor XI activity. A dosage calculation and follow up factor XI assay are necessary to assure proper hemostatic levels.

4. Dosage calculation of fresh frozen plasma (FFP): Calculating the patient's plasma volume and reaching an adequate level of factor XI activity are essential in establishing hemostasis in surgery. A level of 40% is adequate to cover a major surgical procedure. Calculating the dose requires the plasma volume and half-life of the factor in the plasma. Total blood volume is approximately 7% of body weight. Blood volume adjusted for sex, height and weight are available in published tables in textbooks. This patient weighs 50 kg and has a hematocrit of 30%.

   Blood volume = body weight (kg) x 7%
   Blood volume = 50 kg x 0.07
   Blood volume = 3,500 ml
   Plasma volume = blood volume x (1 – hematocrit)
   Plasma volume = 3,500 ml x (1 – 0.30)
   Plasma volume = 2,450 ml

   FFP has 1 U/ml of factor XI activity. Each unit of FFP (250 ml) has approximately 250 units of factor XI activity. For a patient with a plasma volume of 2,450 ml, approximately 980 units of factor XI are necessary to administer to raise the plasma level to 40% (0.4 x 2,450 = 1,960). The calculated dose prior to surgery (980 / 250 = 3.9) is 4 units of FFP. Please note, a hematologist's expertise is necessary in treatment prior to surgery.

   Factor XI assays are not available at Rex Hospital Lab. Turn-around-time for factor XI assays at the Mayo Clinic Lab is 48 hours. Assays for factors VIII, IX and XIII are available routinely at Rex Lab with results available the same day.

5. Patients with mild factor XI disorders (4 to 8% activity) may be able to avoid plasma products when undergoing dental procedures. The use of topical antifibrinolytic drugs such as -aminocaproic acid (Amicar or Immunex) or tranexamic acid combined with aggressive surgical hemostasis is usually all that is required to provide hemostasis. Antifibrinolytics should be avoided in those patients with hematuria because of urinary tract clots may cause obstruction.

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The author is indebted to Dr. Kenneth Zeitler for sharing his expertise in the field of coagulation and valuable suggestions in the preparation of this article.

Reference:
1. Case Studies in Hemostasis, Laboratory Diagnosis and Management, George M Roberts, MD, PhD, American Society of Clinical Pathology Press, 2000, p41 and 118.

Improved SurePath™ Specimen Processing Time

At Rex we have gradually converted from the ThinPrep® technique to the Surepath™ method for our liquid-based, thin layer Pap tests. Both preparations are superior to the conventional Pap smear for disease detection. However, the SurePath™ method uses a density gradient process that excludes extraneous materials such as blood, mucus, inflammation, and lubricant from the resultant Pap slides, significantly reducing the frequency of unsatisfactory specimens when compared to either ThinPrep® or conventional Pap smears. This SurePath™ density gradient enrichment process is more labor intensive but the product is worth the extra effort. We are fortunate at Rex Laboratory to have diligent, creative and hard-working employees such as David Shepard. Mr. Shepard developed a novel procedure for SurePath™ specimen processing, improving efficiency and producing a superior product. Mr. Shepard recently presented this procedure as a poster at the annual meeting of the American Society of Cytopathology in Chicago. A reproduction of his poster is presented on the inserted page.