REX HEALTHCARE

Laboratory Bulletin...

Updates and Information from Rex Healthcare and Rex Outreach

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Changes in Fetal Lung Maturity Evaluation

Effective July 21, 1997, Rex Healthcare Laboratory will modify the approach to amniotic fluid testing for fetal lung maturity. The lecithin/sphingomyelin (L/S) ratio and foam stability index (FSI) will be discontinued. The recommended test will be the fluorescence polarization assay for surfactant (FLM - surfactant) developed for the Abbott TDX.

Background

Long considered the "gold standard" for fetal lung maturity testing, the L/S ratio suffers from being labor intensive, time-consuming, and having poor precision (coefficients of variation in recent College of American Pathologists proficiency survey of 26% and 27%). A recent paper recommended that laboratories discontinue L/S ratio testing if they receive less than 15 specimens per week.[1] (Our laboratory averages 1 per week.) The number of laboratories performing the L/S ratios is declining steadily.

In April of this year, Beckman Instruments, Inc. announced that "as a result of routine assessments", they would discontinue manufacture of their ubiquitous Lumadex-FSI foam stability index assay.[2] This test, though elegant in principle, requires a moderate degree of skill to provide reproducible results.

The FLM-surfactant assay developed by Abbott has become increasingly popular (probably contributing to the decline of the L/S ratio and FSI.) The test is automated, relatively rapid, and has a high degree of precision. It is considered the best rapid test for fetal lung maturity.[1] A result >54 mg surfactant/g protein indicates lung maturity. A result <40 mg/g predicts immaturity. Results between 40-54 mg/g are considered "transitional". The results are not affected by the presence or absence of maternal diabetes. It is subject to interference by blood, meconium, maternal urine, or vaginal secretions. Specimens contaminated by these materials should be assayed by another method.

Two other tests deserve brief mention. The phosphatidyl glycerol (PG) slide test is rapid, precise, and is not affected by contamination with blood or vaginal secretion. A positive result virtually assures fetal lung maturity (high specificity). The downside is the legendary lack of sensitivity. In a retrospective review of Rex Healthcare Laboratory data,[3] a positive PG was encountered in only 18.5% of specimens interpreted as "mature" or "low risk of Respiratory Distress Syndrome"

by either L/S ratio, FSI, FLM, or some combination of the above. There were no cases where the PG was the only test to indicate maturity when the other tests did not.

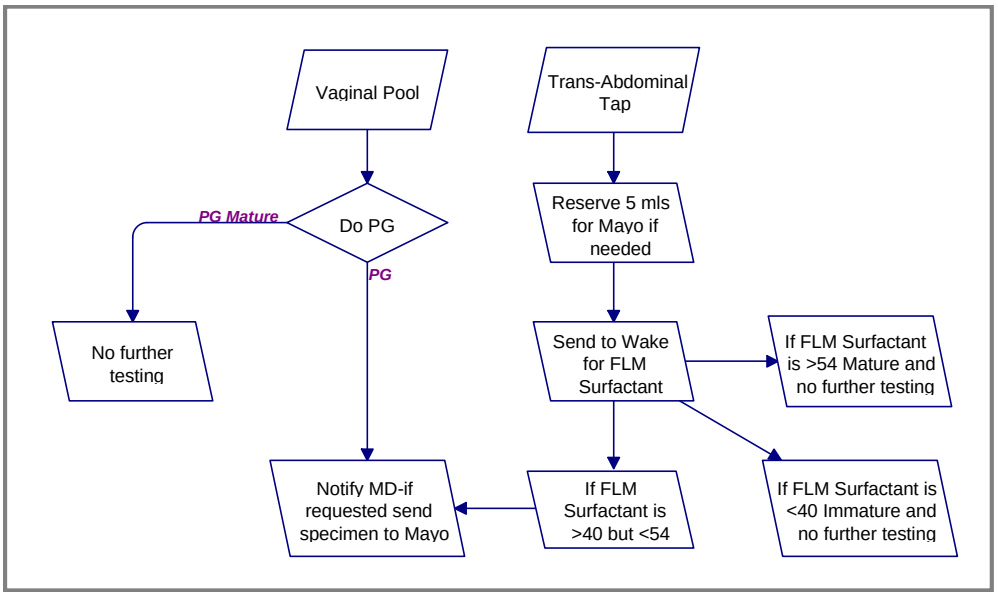
Lamellar body (LB) counts are extremely rapid, automated, quantitative and precise. In a retrospective study of Rex Laboratory data,[3] they correlated well with the L/S ratio, FSI, and FLM. (Indeed, the LB's appeared to correlate as well as or better than the other 3 tests did among themselves!) However, this assay is still "relatively new" and regarded as "investigational" by organized obstetrical organizations and one local expert.[4,5] For the present time, LB counts should probably be interpreted in conjunction with other test results.

Testing Strategy Developed

To improve efficiency and lower cost, the Rex Healthcare Department of Obstetrics & Gynecology (in collaboration with the Pathology Laboratory) has developed the following testing strategy for fetal lung maturity assessment on amniotic fluid.

Transabdominal amniocentesis: Collect 10 ml of uncontaminated amniotic fluid. Label appropriately, place on ice. Order "FLM-Surfactant" in hospital computer (initially this may have to be ordered as a "Reference" test, but direct entry will become available in the near future). A "lamellar body" study may also be ordered at the discretion of the attending obstetrician. Deliver the specimen promptly to the Laboratory. An aliquot will be forwarded to Wake Medical Center Pathology Laboratory for the FLM-Surfactant assay. The results will be phoned to the Birth Center nursing station (or physician's office for outpatients). A result >54 mg/g is "mature" while a result <40 mg/g is "immature". No further testing is required. Results between 40-54 are "transitional". If further testing is desired, the physician may direct the Rex Laboratory to forward a second aliquot to Mayo Medical Laboratories for an L/S ratio.

Ruptured Amniotic Membranes: Vaginal pool samples are frequently contaminated with blood, mucus, and bacteria. They are not acceptable for FLM-surfactant testing. The obstetrician may consider ultrasound to identify pockets of residual amniotic fluid for amniocentesis and testing by the above algorithm. Vaginal pool specimens will be tested by the rapid PG slide test. If this is positive, there will be no further testing. If this is negative, the physician may direct the Laboratory to forward any remaining specimen to Mayo Medical Laboratories for an L/S ratio.



Questions or concerns are welcome and should be directed to Dr. Benson (783-3059) or Dr. Robert Brainard (783-3056).

John D. Benson, M.D.
Jerome B. Gardner, M.D.

References:

1. Ashwood ER. Standards of laboratory practice: evaluation of fetal lung maturity. Clin Chem 43:211-4, 1997.
2. Beckman Diagnostics. Notice of Product Discontinuance (Lumadex-FSI P/N 667790), 4/23/97.
3. Benson JD. Fetal Lung Maturity Studies (Rex Healthcare 9/6/95 - 6/4/97). On file.
4. American College of Obstetrics & Gynecology Educational Bulletin #230, Nov. 1996.
5. Herbert W. (Personal communication to Jerome Gardner, MD)

Rocky Mountain Spotted Fever

There is a dearth of available laboratory procedures for the early diagnosis of Rocky Mountain Spotted Fever. Various serological tests can be used to confirm the diagnosis of Rocky Mountain Spotted fever, but, in general, the antibodies are not detectable until several days or more after the onset of illness. Obviously, such a delay is unacceptable for the adequate treatment of Rocky Mountain Spotted Fever. In the past, a specific test to diagnose Rocky Mountain Spotted Fever in its early stages was a direct immunofluorescent examination of the skin biopsy samples. These samples were examined for *R. rickettsii* antigen using antibodies supplied by the Centers for Disease Control and Prevention (CDC). However, the CDC is no longer supplying such an antibody to any clinical laboratories, and therefore the test is no longer available. The Weil-Felix assay, which measures the agglutination of OX-19 and OX-2 antigen of proteus vulgaris, lacks sensitivity and specificity and is no longer recommended. Culture of rickettsiae requires specific conditions and is rarely performed. Although not available for clinical diagnostic purposes, the polymerase chain reaction technique has been used to detect *R. rickettsii* DNA in blood samples from patients with severe disease. Perhaps this may prove to be a clinically useful procedure in the future, but such techniques are not currently available. In most situations, clinicians must rely on the recognition of the early signs of Rocky Mountain Spotted Fever and initiate therapy without laboratory confirmation.

The period between tick bite and the development of clinical symptoms ranges from 2 to 14 days. The disease usually begins with a fever, myalgia and headache. A variety of other symptoms may occur including nausea, vomiting and abdominal pain. The rash, the major diagnostic sign, appears in a small fraction of patients on the first day of the disease and in only 49 percent during the first 3 days, usually appearing 3-5 days after the onset of fever and occurring in 84-91 percent of patients. Disease without a rash occurs more often in older patients and in black patients. The rash typically begins around the wrists and ankles but may start on the trunk. Involvement of the palms and soles is considered characteristic, yet occurs in only 36-82 percent of patients who have a rash and often appears late in the course.[1]

1. *Principles and Practice of Infectious Diseases*, Mandell, Bennett and Dolin, 1995.

Robert E. Kanich, M.D.

Changes in DNA cell cycle analysis and Helper/ suppressor ratios (CD4/ CD8)

Effective Monday, August 11, 1997, the flow cytometer in the Rex Cancer Center Laboratory will cease operation. The new methodology for Helper T cell CD4 counts will now be fluorospheres (a Coulter developed antibody bead technology) on the Coulter STK-S cell counter rather than dual-color immunophenotyping on the flow cytometer. The new fluorosphere method is a direct count method for CD4 (and CD8 if requested). In published literature studies, the two methods are very highly correlated. Regression analysis comparison between the two methods gave correlation coefficients (R^2) in the range 0.99 to 0.97. As part of this change over, we will measure patients by both methods for the next month to ensure continuity of values and establish a new baseline for patients. The laboratory will be performing only absolute CD4 counts unless you specifically request CD8 count and the CD4/CD8 ratio.

The specimen requirement for the new CD4 method is EDTA whole blood, one full 5 ml EDTA (lavender top) tube. The handling and stability requirements remain the same, specimen kept at room temperature and should arrive in the laboratory the same day it was drawn.

Specimens for DNA Cell Cycle Analysis will be forwarded to Cytometry Associates who will perform the analysis and bill the patient's insurance directly. We currently use Cytometry Associates for leukemia immunophenotyping. Specimen handling and expected turnaround time will remain the same. Requests for DNA analysis of tissue will have to be made for each specific case. The laboratory will no longer assume that any particular case or type of cases merit DNA analysis.

Robert B. Brainard, Ph.D.

Yeast susceptibility data

Rex Hospital Laboratory does not currently perform susceptibility testing on yeast. Dr. Lizzie Harrell at Duke has provided us with data from Duke for 1996. This is reprinted with permission below.

Karl T. Kleeman, Ph.D.

Yeast: In-Vitro Susceptibility to Antifungal Drugs

	Amphotericin B	Fluconazole
Candida albicans	●	●
C. tropicalis, C. parapsilosis	●	⊙
C. krusei, T. glabrata	●	○

C. lusitaniae	○		
Cryptoccoccus neoformans	●		●

● = usually susceptible ⊙= susceptibility varies ○= often resistent

Testing is useful in serious or persistent infections due to organisms with unpredictable susceptibility profiles, especially when standard regimens fail or are not possible.

For further information, call the Laboratory (783-3040). Telephone extensions are: Dr. Benson (3059), Dr. Brainard (3056), Dr. Carter (3058), Dr. Chiavetta (3040), Dr. Kanich (3057), Dr. Kleeman (3063), Dr. Nance (3286), Dr. Sorge (3062), Robin Ivosic (Core Lab Manager 3053), Linda Lompa (Blood Services Manager 785-4770), Kimberly Skelding (Customer Services Manager 3318), Rex Outreach (783-3040), Karen Sanderson (Lab Compliance Specialist 3396).