Standards of laboratory practice: evaluation of fetal lung maturity

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In this standard of laboratory practice I recommend guidelines for fetal lung maturity (FLM) testing. If possible, obtain a 10-mL uncontaminated sample by amniocentesis. Keep the amniotic fluid at 4°C and mix well before testing. If centrifugation is required, strictly adhere to the protocol. Most laboratories should offer a rapid test, such as fluorescence polarization, phosphatidylglycerol, or foam stability index, daily on both a routine and emergency basis. Requests for lecithin/sphingomyelin ratio may be referred to a reference laboratory. Communicate immediately the results of any FLM test to the ordering location. The report should contain the result, sample contamination, and reference information. Separate reference intervals for diabetic patients are not recommended.

INDEXING TERMS: guidelines • fetal organ maturity • amniotic fluid chemistry • phosphatidylglycerol • lecithin/sphingomyelin ratio • fluorescence polarization

This document provides guidelines to laboratories involved in evaluating fetal lung maturity (FLM) for the prevention of respiratory distress syndrome (RDS). Patient populations can vary significantly across differing healthcare centers. This variation affects the requirements for turnaround time and test predictability, and therefore the selection of the “best” method is dependent on the healthcare setting. Methods that work well in low-risk full-term pregnancies often perform poorly when used in high-risk preterm pregnancies. The guidelines include preanalytical considerations (sample collection, condition, and handling), analytical approaches (techniques, turnaround time, sample requirements, effect of contamination), and interpretation of results (reference intervals, prediction of fetal lung status, effect of differing patient populations). I review lecithin/sphingomyelin (L/S) ratio [1, 2], fluorescence polarization (FP) [3–6], phosphatidylglycerol (PG) [7], foam stability index (FSI) [8], and lamellar body counts (LBC) [9–11]. I have purposefully avoided tests that do not measure pulmonary surfactant (e.g., creatinine, lipid-staining cells) and those novel tests that, while promising, do not have a broad enough acceptance or enough prospective studies to yet justify their recommendation (e.g., refractive index-matched anomalous diffraction [12], nuclear magnetic resonance spectroscopy [13]).

Preanalytical Considerations

SAMPLE COLLECTION AND CONDITION

Although the laboratory has little control over amniotic fluid sample collection, cooperation from the clinician collecting the sample can lead to improved test results. I recommend that the sample be obtained by amniocentesis and consist of at least 10 mL of uncontaminated amniotic fluid. The physician performing the procedure should make every attempt to avoid traversing the placenta. If the placenta is located in an anterior position, this may be unavoidable and the sample is likely to be contaminated with blood. Blood contamination affects most of the FLM tests because plasma contains a high concentration of most phospholipids. The exception is PG, which is absent from plasma. For L/S ratio and FP, the result tends to move toward a transitional value. In other words, mature results become less mature and immature results become more mature. FSI can be falsely mature and LBC can be falsely immature when blood contamination is present. If bloody fluid is evident during the amniocentesis, multiple samplings are recommended. The sample least bloody should be sent for FLM testing.

During amniocentesis, the location of the needle will affect the result of the FLM test. Pulmonary surfactant concentration will be higher in the fluid surrounding the fetal mouth. Thus, samples obtained close to the fetal head will be more mature than those obtained at other
sites. Because this effect is subtle, I recommend that FLM results not be interpreted with respect to location of specimen acquisition.

Samples obtained from the vaginal posterior fornix after rupture of the membranes are commonly contaminated with blood, bacteria, and mucus. Vaginal pool samples are adequate for testing only when the fluid has been in the vagina a short time and the sample is quickly chilled after collection [14]. I recommend that the clinician seriously consider amniocentesis in patients with ruptured membranes. Often, a pocket of amniotic fluid can be located by ultrasound and collected by amniocentesis without endangering the fetus. This type of sample is much preferred over a vaginal pool sample.

Amniocentesis trays generally contain an amber collection tube. While this amber tube is satisfactory for the collection of FLM specimens, it is not required. The phospholipids present in amniotic fluid are not light sensitive. The amber tube is required if the measurement of amniotic fluid bilirubin is anticipated, as in a case of erythroblastosis fetalis.

After collection, I recommend that the specimen tube be immersed in wet ice for transportation to the laboratory. Chilling the sample reduces the activity of any enzymes that might be present and could degrade the phospholipids.

**SAMPLE HANDLING**

Most samples for FLM assessment are tested the day of collection. When testing must be delayed, refrigeration at 4°C is appropriate. Before testing, samples should be mixed well, assessed for contamination, and, if required by the method, centrifuged. For long-term studies, centrifuged amniotic fluid can be stored at −20°C or −70°C.

Surfactant phospholipids are present in amniotic fluid in fairly large particles, lamellar bodies. These bodies are ~0.5–1.0 μm in diameter [15]. Their density is greater than that of the aqueous phase of amniotic fluid. Therefore, the lamellar bodies will settle to the bottom of the collection container if the specimen is allowed to stand undisturbed. Samples must be thoroughly mixed before splitting, aliquoting, or testing. Vortex-mixing should be avoided because it causes the samples to foam, especially when the samples are mature. I recommend that amniotic fluid samples be mixed by placing them on a hematology tube rocker for not less than 2 min. The sample should not be allowed to stand after mixing, but instead immediately processed.

A note should be made if the sample contains blood, meconium, bilirubin, or mucus. This can be best assessed if the entire sample is decanted from the usually amber collection tube into a new clear specimen tube. The results of most methods will be affected by a 1% or greater contamination with blood. The best way to visually estimate this amount of contamination is to add 10 μL of EDTA–whole blood to a 1-mL amniotic fluid specimen for comparison. Green-tinged fluid indicates the presence of meconium or old blood. If heavily contaminated, the specimen will contain a large amount of particulate matter. Specimens with a yellow tint indicate the presence of bilirubin. Many vaginal pool specimens will contain mucus, which can be assessed while decanting from the collection tube into the new specimen tube.

If the analytical method requires a centrifugation step, then great care should be taken to follow the protocol exactly. My laboratory uses 400g for 2 min. This will remove red blood cells, large clumps of mucus, and, unfortunately, ~8% of the lamellar bodies. Always recalculate the centrifugal speed needed to achieve the required centrifugal force when using a new centrifuge or a different rotor. Longer centrifugation periods and higher forces remove more surfactant.

**Analytical Considerations**

**METHOD SELECTION**

Although many clinicians consider the L/S ratio to be the gold standard in FLM testing, hardly any laboratorians agree. In many healthcare settings, the clinicians have come to rely on tests other than L/S ratio. In other settings, the clinicians will demand the L/S ratio regardless of the other tests available. The performance and interpretation of the L/S ratio requires technologist expertise that is achieved only by performing the test frequently. The method is labor-intensive, taking 3–5 h to perform. Precision is poor even with uniform artificial amniotic fluid samples, as shown by a recent College of American Pathologists (CAP) proficiency survey (sample identification, mean, SD, and CV): LM-09, 4.46, 1.18, 26%; and LM-10, 2.31, 0.62, 27% [16]. I recommend that laboratories do not perform L/S ratio testing if they receive <15 requests per week. These samples should be sent to a reference laboratory for L/S ratio testing if the clinicians feel that they must have this test. For laboratories receiving ≥15 L/S ratio requests per week, I recommend either performing the test in-house or referring it to a reference laboratory. The Helena method for L/S ratio testing is a satisfactory one-dimensional method.

All laboratories that support obstetric patients should offer at least one test that can be performed in <1 h. The choices are: the FP method on the TDx (either with the Abbott TDx/FLM® test, which reports in milligram of surfactant per gram of albumin, or the home-brew version that involves fluorescently labeled phosphatidylcholine and has units of polarization), PG with the slide method (AmnioStat-FLM® from Irvine Scientific), or the foam stability test (either with the Beckman Lumadex®-FSI, or a home-brew version that involves ethanol and amniotic fluid mixtures at differing concentrations).

The FP method on the TDx is the best rapid test in a high-risk setting. This test can be easily offered 24 h per day, every day of the week. The results are quantitative and changes in the results indicate changes in the risk of RDS. Precision is good as shown by a recent CAP proficiency survey (sample identification, mean, SD, and CV):
LM-11, 114.7 mg/g, 5.7 mg/g, 5.0%; and LM-12, 19.9 mg/g, 0.7 mg/g, 3.9% [16]. The Abbott and home-brew FP methods produce equivalent results [6].

The Irvine Scientific slide test for PG works best in a low-risk population because of the poor predictive value of an immature result. A disadvantage of this test is the qualitative nature of the result: negative, weakly positive, or positive. Thus, with a negative (immature) result, a clinician will not be able to determine if the fetal lungs have started to mature. A major advantage is that PG is unaffected by blood contamination. Accuracy is good as assessed by a recent CAP proficiency survey (sample identification and percent correctly classified): LM-09, 98.6% positive; and LM-10, 93.9% negative [16].

Finally, laboratories should consider offering LBC [9–11]. This test takes <15 min and is performed by counting particles in the platelet channel of a hematology blood analyzer. Most studies have used a Coulter Counter. The results may differ on other brands of blood cell counters because of size interval differences in the platelet counting channel. Because of the small number of prospective clinical outcome studies reported, the results of this test should be reported in parallel with one of the rapid tests above.

**FREQUENCY OF TESTING**

Laboratories performing L/S ratio should offer routine testing once per day, 7 days per week. Emergency requests for this test should be discussed with the laboratory director or supervising technologist. All other FLM tests should be performed daily in a routine run and be available at any time for emergency testing without prior approval. The turnaround time for these rapid tests should be <1 h.

**Postanalytical Considerations**

**COMMUNICATION OF RESULTS**

Results of FLM tests should be immediately communicated to the ordering location. Even though the sample may have been obtained several hours before testing, the result plays an important role in the clinician’s decision to accelerate delivery, observe, or actively delay birth. The report of the FLM test should include the test result, sample condition if bloody or meconium-stained, and reference information.

**INTERPRETATION OF RESULTS**

L/S methods can vary substantially. Therefore, laboratories performing L/S ratio testing should either conduct a clinical outcome study to validate their reference intervals or compare their method with one in the literature that does have a clinical outcome study. The FP test on the TDx, the slide-based PG, and the FSI are better standardized than the L/S ratio. Laboratories should use with confidence the literature-based reference information for these rapid tests.

Care should be taken to avoid misrepresenting the predictive value of a mature or immature test. Most recent clinical outcome studies appropriately evaluate the FLM test results by using a ROC curve [17]. This is a plot of the sensitivity (percent of affected cases having an immature result) vs 1 – specificity (percent of unaffected cases having an immature result). The predictive value of any given result is strongly affected by the prevalence of respiratory distress for that healthcare facility. The prevalence can vary from 3% to 15% depending on the acuity of the patients. Thus, statements regarding the predictive value of a mature test may be accurate for one healthcare setting and quite biased in another. Logistical models to predict the risk of RDS have been proposed that include the FLM result and the gestational age [18, 19]. Theoretically, these models include gestational age to represent both the prior probability of RDS and other factors that affect the FLM result. If these models are confirmed by other investigators, they may be useful for interpreting FLM results across differing healthcare settings.

The dogma in FLM testing teaches that diabetes delays pulmonary maturation and makes the FLM test results unreliable. This belief comes from older studies that showed immature FLM test results in term diabetic pregnancies. Pregnancy management for patients with diabetes has, in the past decade, emphasized tight glycemic control. Poorly controlled diabetes clearly delays pulmonary maturation [20]. Well-controlled diabetes very likely does not delay maturation nor make the FLM test results unreliable. There is no clinical outcome study in the literature with enough RDS cases from diabetic mothers to prove that the tests are unreliable. There is indirect evidence that the FP test is reliable [5, 19, 21]. Thus, I recommend against using separate reference intervals for diabetic patients.

**References**

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