

## CHAPTER

## 14

# Amniotic Fluid

## LEARNING OBJECTIVES

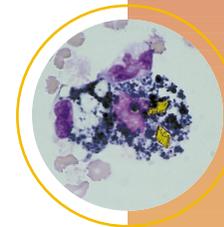
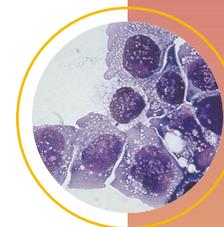
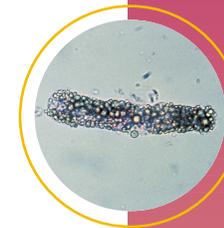
Upon completion of this chapter, the reader will be able to:

- 1 State the functions of amniotic fluid.
- 2 Describe the formation and composition of amniotic fluid.
- 3 Describe the specimen handling and processing procedures for testing of amniotic fluid for bilirubin, fetal lung maturity (FLM), and cytogenetic analysis.
- 4 Discuss the principle of the spectrophotometric analysis for evaluation of hemolytic disease of the newborn.
- 5 Interpret a Liley graph.
- 6 Describe the analysis of amniotic fluid for the detection of neural tube disorders.
- 7 Explain the physiologic significance of the lecithin-sphingomyelin (L/S) ratio.
- 8 State the relationship of phosphatidyl glycerol to FLM.
- 9 Discuss the principle of and sources of error for the L/S ratio, Amniostat-FLM, Foam Stability Index, and microviscosity tests for FLM.
- 10 Describe the relationship of lamellar bodies to FLM and the laboratory tests performed.

## KEY TERMS

amniocentesis  
 cytogenetic analysis  
 fetal lung maturity  
 hemolytic disease of the newborn

lamellar body  
 lecithin-sphingomyelin ratio  
 surfactants



Although the testing of amniotic fluid is frequently associated with **cytogenetic analysis**, the clinical laboratory also performs several significant tests on amniotic fluid. Because amniotic fluid is a product of fetal metabolism, the constituents that are present in the fluid provide information about the metabolic processes taking place and the progress of fetal maturation. When conditions that adversely affect the fetus arise, the danger to the fetus must be measured against the ability of the fetus to survive an early delivery. The tests covered in this chapter are used to determine the extent of fetal distress and fetal maturity (Table 14–1).

## Physiology

Amniotic fluid is present in the **amnion**, a membranous sac that surrounds the fetus (Figure 14–1). The primary function of the fluid is to provide a protective cushion for the fetus and allow movement. Exchanges of water and chemicals also take place between the fluid, the fetus, and the maternal circulation.

The amount of amniotic fluid increases throughout pregnancy, reaching a peak of approximately 1 L during the third trimester, and then gradually decreases prior to delivery. During the first trimester, the approximately 35 mL of amniotic fluid is derived primarily from the maternal circulation. The fluid has a composition similar to that of the maternal plasma and contains a small amount of sloughed fetal cells. These cells provide the basis for cytogenetic analysis.

After the first trimester, fetal urine is the major contributor to the amniotic fluid volume. At the time that fetal urine production occurs, fetal swallowing of the amniotic fluid begins and regulates the increase in fluid from the fetal urine. Failure of the fetus to begin swallowing results in excessive accumulation of amniotic fluid (**hydramnios**) and is an indication of fetal distress, often associated with neural tube disorders. Increased fetal swallowing, urinary tract deformities, and membrane leakage are possible causes of decreased amniotic fluid (**oligohydramnios**).

As would be expected, the chemical composition of the amniotic fluid changes when fetal urine production begins.

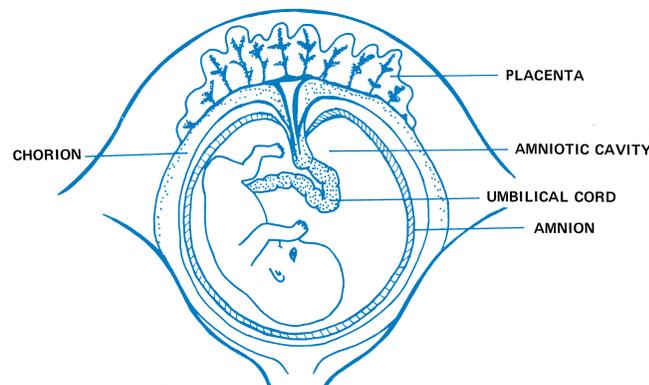


FIGURE 14–1 Fetus in amniotic sac.

The concentrations of creatinine, urea, and uric acid increase, whereas glucose and protein concentrations decrease. Concentrations of electrolytes, enzymes, hormones, and metabolic end products also vary but are of little clinical significance. Measurement of amniotic fluid creatinine has been used to determine fetal age. Prior to 36 weeks' gestation, the amniotic fluid creatinine level ranges between 1.5 and 2.0 mg/dL. It then rises above 2.0 mg/dL, thereby providing a means of determining fetal age as greater than 36 weeks.<sup>15</sup>

Differentiation between amniotic fluid and maternal urine may be necessary to determine possible premature membrane rupture or accidental puncture of the maternal bladder during specimen collection. Chemical analysis of creatinine, urea, glucose, and protein will aid in the differentiation. Levels of creatinine and urea are much lower in amniotic fluid than in urine. Creatinine does not exceed 3.5 mg/dL and urea 30 mg/dL in amniotic fluid, whereas values as high as 10 mg/dL for creatinine and 300 mg/dL for urea may be found in urine.<sup>16</sup> Measurement of glucose and protein is a less reliable indicator, because glucose and protein are not uncommon urine constituents during pregnancy. However, under normal circumstances, the presence of glucose, protein, or both is associated more closely with amniotic fluid.

TABLE 14–1 Tests for Fetal Well-Being and Maturity

Test	Normal Values at Term <sup>16</sup>	Significance
Bilirubin scan	$\Delta A_{450} > .025$	Hemolytic disease of the newborn
Alpha-fetoprotein	$< 2.0 \text{ MoM}$	Neural tube disorders
Lecithin-sphingomyelin ratio	$\geq 2.0$	Fetal lung maturity
Amniostat-fetal lung maturity	Positive	Fetal lung maturity/phosphatidyl glycerol
Foam Stability Index	$\geq 47$	Fetal lung maturity
Microviscosity	$\geq 70 \text{ mg/g}$	Fetal lung maturity
Optical density 650 nm	$\geq 0.150$	Fetal lung maturity
Lamellar body count	$\geq 32,000/\mu\text{L}$	Fetal lung maturity

## Specimen Collection

Amniotic fluid is obtained by needle aspiration into the amniotic sac, a procedure called **amniocentesis**. The procedure most frequently performed is a transabdominal amniocentesis. Vaginal amniocentesis may also be performed; however, this method carries a greater risk of infection. In general, amniocentesis is a safe procedure, particularly when performed after the 14th week of gestation. Fluid for chromosome analysis is usually collected at approximately 16 weeks' gestation, whereas tests for fetal distress and maturity are performed later in the third trimester.

A maximum of 30 mL of amniotic fluid is collected in sterile syringes. The first 2 or 3 mL collected can be contaminated by maternal blood, tissue fluid, and cells and are discarded. Fluid for bilirubin analysis in cases of **hemolytic disease of the newborn (HDN)** must be protected from light at all times. This can be accomplished by placing the specimens in amber-colored tubes.

## Specimen Handling and Processing

Handling and processing of amniotic fluid vary with the tests requested. However, in all circumstances, special handling procedures should be performed immediately and the specimen delivered promptly to the laboratory. Fluid for **fetal lung maturity (FLM)** tests should be placed in ice for delivery to the laboratory and refrigerated prior to testing. Specimens for cytogenetic studies are maintained at room temperature or body temperature (37°C incubation) prior to analysis to prolong the life of the cells needed for analysis.

All fluid for chemical testing should be separated from cellular elements and debris as soon as possible to prevent distortion of chemical constituents by cellular metabolism or disintegration. This can be performed using centrifugation or filtration. Low-speed centrifugation (500 to 1000 g) for no longer than 5 minutes is required for FLM testing, because at higher speeds some of the phospholipids measured in the tests may be lost in the sediment. Filtration is often recommended for FLM methods to prevent loss of the phospholipids.

## Color and Appearance

Normal amniotic fluid is colorless and may exhibit slight to moderate turbidity from cellular debris, particularly in later stages of fetal development. Blood-streaked fluid may be present as the result of a traumatic tap, abdominal trauma, or intra-amniotic hemorrhage. The source of the blood (maternal or fetal) can be determined using the Kleihauer-Betke test for fetal hemoglobin and is important for further case management.

The presence of bilirubin gives the fluid a yellow color and is indicative of red blood cell destruction resulting from HDN. **Meconium**, which is usually defined as a newborn's first bowel movement, may be present in the amni-

### Summary of Amniotic Fluid Color

Color	Significance
Colorless	Normal
Blood-streaked	Traumatic tap, abdominal trauma, intra-amniotic hemorrhage
Yellow	Hemolytic disease of the newborn (bilirubin)
Dark green	Meconium
Dark red-brown	Fetal death

otic fluid as the result of fetal intestinal secretions. It produces a dark green color. Fetal aspiration of meconium during fetal swallowing is a concern when increased amounts are present in the fluid. A very dark red-brown fluid is associated with fetal death.

## Tests for Fetal Distress

### HEMOLYTIC DISEASE OF THE NEWBORN

The oldest routinely performed laboratory test on amniotic fluid evaluates the severity of the fetal anemia produced by HDN. The incidence of this disease has been decreasing rapidly since the development of methods to prevent anti-Rh antibody production in postpartum mothers. However, antibodies against other red cell antigens are also capable of producing HDN, and immunization of Rh-negative mothers may not be effective or even performed in all cases. When antibodies present in the maternal circulation cross the placenta, the destruction of fetal red blood cells results in the appearance of the red blood cell degradation product, bilirubin, in the amniotic fluid. By measuring the amount of bilirubin in the fluid, the degree of hemolysis taking place may be determined and the danger this anemia presents to the fetus may be assessed.

The measurement of amniotic fluid bilirubin is performed by spectrophotometric analysis. As illustrated in Figure 14-2, the optical density (OD) of the fluid is mea-

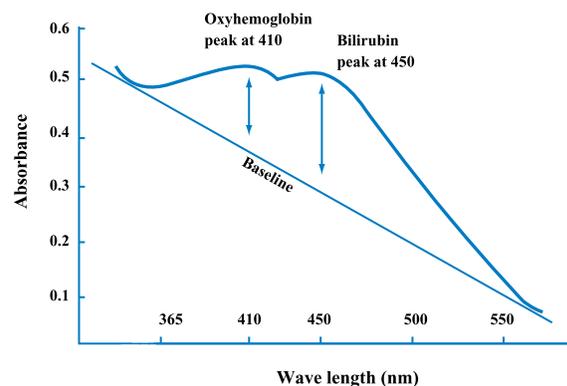


FIGURE 14-2 Spectrophotometric bilirubin scan showing bilirubin and oxyhemoglobin peaks.

sured in intervals between 365 nm and 550 nm and the readings plotted on semilogarithmic graph paper. In normal fluid, the OD will be highest at 365 nm and decrease linearly to 550 nm. When bilirubin is present, a rise in OD will be seen at 450 nm because this is the wavelength of maximum bilirubin absorption. The difference between the OD of the theoretic baseline and the OD at 450 nm represents the amniotic fluid bilirubin concentration. This difference in OD, referred to as the absorbance difference at 450 nm ( $\Delta A_{450}$ ), is then plotted on a Liley graph to determine the severity of the hemolytic disease (Figure 14-3).<sup>9</sup>

Notice that the Liley graph plots the  $\Delta A_{450}$  against gestational age and is divided into three zones that represent the degree of hemolytic severity. Values falling in zone I indicate no more than a mildly affected fetus; those in zone II require careful monitoring, whereas a value in zone III suggests a severely affected fetus. Intervention through induction of labor or intrauterine exchange transfusion must be considered when a  $\Delta A_{450}$  is plotted in zone III.

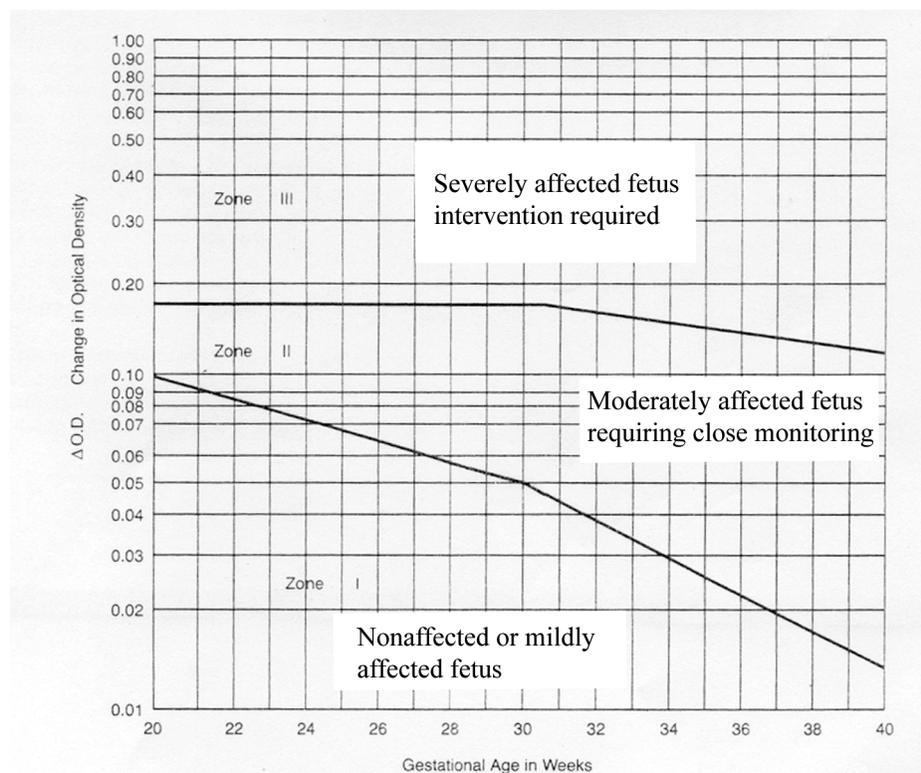
As mentioned previously, specimens must be protected from light at all times. Markedly decreased values will be obtained with as little as 30 minutes of exposure to light. Care must be taken to ensure that contamination of the fluid by cells, hemoglobin, meconium, or other debris does not interfere with the spectrophotometric analysis. Specimens should be immediately centrifuged to remove particulate interference. Maximum absorbance of oxyhemoglobin occurs at 410 nm and can interfere with the bilirubin absorption peak (see Fig. 14-2). This interference can be removed by extraction with chloroform if necessary.<sup>13</sup> A

control may be prepared by diluting commercial chemistry control sera 1 to 10 with normal saline and treating it in the same manner as the patient specimen. Bilirubin and protein levels approximate those in amniotic fluid and can be varied by using low or high control sera.<sup>10</sup>

## NEURAL TUBE DEFECTS

Increased levels of alpha-fetoprotein (AFP) in both the maternal circulation and the amniotic fluid can be indicative of fetal neural tube defects, such as anencephaly and spina bifida. AFP is the major protein produced by the fetal liver during early gestation (prior to 18 weeks). It is found in the maternal serum due to the combined fetal-maternal circulations and in the amniotic fluid from diffusion and excretion of fetal urine. Increased levels are found in the maternal serum and amniotic fluid when the skin fails to close over the neural tissue, as occurs in anencephaly and spina bifida.

Measurement of amniotic fluid AFP levels is indicated when maternal serum levels are elevated or a family history of previous neural tube defects exists. The possibility of a multiple pregnancy also must be investigated when serum levels are elevated. Normal values are based on the week of gestational age, as the fetus produces maximal AFP between 12 and 15 weeks' gestation, after which levels in amniotic fluid then begin to decline. Both serum and amniotic fluid AFP levels are reported in terms of multiples of the median (MoM). The median is the laboratory's reference level for a given week of gestation. A value two times



**FIGURE 14-3** Example of a Liley graph. (Adapted from Harming, DM: Modern Blood Banking and Transfusion Practices, ed 4. FA Davis, Philadelphia, 1999, p. 427)

the median value is considered abnormal (greater than two MoM) for both maternal serum and amniotic fluid. Testing for AFP has been automated by the Access Immunoassay System (Beckman Coulter, Inc., Fullerton, CA.).

Elevated amniotic fluid AFP levels are followed by measurement of amniotic acetylcholinesterase (**AChE**). The test is more specific for neural tube disorders than AFP, provided it is not performed on a bloody specimen, because blood contains AChE.<sup>16</sup>

## Tests for Fetal Maturity

Fetal distress, whether caused by HDN or other conditions, forces the obstetrician to consider a preterm delivery. At this point, fetal maturity must be assessed.

### FETAL LUNG MATURITY

Respiratory distress is the most frequent complication of early delivery. Therefore, laboratory tests must be performed to determine the maturity of the fetal lungs. Several laboratory tests are available to measure FLM.

### LECITHIN-SPHINGOMYELIN RATIO

The reference method to which tests of FLM are compared is the *lecithin-sphingomyelin (L/S) ratio*. Lecithin is the primary component of the *surfactants* (phospholipids, neutral lipids, and proteins) that make up the alveolar lining and account for alveolar stability.

Lecithin is produced at a relatively low and constant rate until the 35th week of gestation, at which time a noticeable increase in its production occurs, resulting in the stabilization of the fetal lung alveoli. Sphingomyelin is a lipid that is produced at a constant rate after about 26 weeks' gestation; therefore, it can serve as a control on which to base the rise in lecithin. Both lecithin and sphingomyelin appear in the amniotic fluid in amounts proportional to their concentrations in the fetus.<sup>7</sup> Prior to 35 weeks' gestation, the L/S ratio is usually less than 1.6 because large amounts of lecithin are not being produced at this time. It will rise to 2.0 or higher when lecithin production increases. Therefore, when the L/S ratio reaches 2.0, a preterm delivery is usually considered to be a relatively safe procedure. Falsely elevated results are encountered in fluid contaminated with blood or meconium because both these substances contain lecithin and sphingomyelin.

Quantitative measurement of lecithin and sphingomyelin is performed using thin-layer chromatography. The procedure is labor intensive and subject to high coefficients of variation. Many laboratories have replaced the L/S ratio with the more cost-effective phosphatidyl glycerol immunoassays, fluorescence polarization, and *lamellar body* density procedures.<sup>4</sup>

### AMNIOSTAT-FLM

The presence of another lung surface lipid, phosphatidyl glycerol, is also essential for adequate lung maturity. The

production of phosphatidyl glycerol normally parallels that of lecithin, but its production is delayed in diabetic mothers. In this circumstance, respiratory distress will occur in the presence of an L/S ratio of 2.0. Therefore, a thin-layer chromatography lung profile must include lecithin, sphingomyelin, and phosphatidyl glycerol to provide an accurate measurement.<sup>8</sup>

Development of an immunologic agglutination test for phosphatidyl glycerol has provided a more rapid method for assessment of fetal maturity that does not require a laboratory to be equipped to perform thin-layer chromatography. The Aminostat-FLM (Irving Scientific, Santa Ana, CA) uses antisera specific for phosphatidyl glycerol and is not affected by specimen contamination with blood and meconium.<sup>5</sup> Studies have shown good correlation with thin-layer chromatography but with a slightly higher incidence of false-negative results that may need to be followed up with further testing.<sup>3,11</sup>

### FOAM STABILITY

Until the development of biochemical techniques to measure the individual lung-surface lipid concentrations, a mechanical screening test, called the "foam" or "shake" test, was used to determine their presence. Because it can be performed at the bedside or in the laboratory, the test is still in use. Amniotic fluid is mixed with 95 percent ethanol, shaken for 15 seconds, and then allowed to sit undisturbed for 15 minutes. At the end of this time, the surface of the fluid is observed for the presence of a continuous line of bubbles around the outside edge. The presence of bubbles indicates that a sufficient amount of phospholipid is available to reduce the surface tension of the fluid even in the presence of alcohol, an antifoaming agent.

A modification of the foam test uses 0.5 mL of amniotic fluid added to increasing amounts of 95 percent ethanol, providing a gradient of ethanol/fluid ratios ranging from 0.42 mL to 0.55 mL in 0.01-mL increments, which can be used to provide a semiquantitative measure of the amount of surfactant present. A value of 47 or higher indicates FLM. The Foam Stability Index has shown good correlation with the L/S ratio and tests for phosphatidyl glycerol. The test cannot be used with contaminated amniotic fluid because blood and meconium will also reduce surface tension.

### PROCEDURE

#### Foam Shake Test Procedure

- 1 Mix equal parts of amniotic fluid with 95% ethanol.
- 2 Vigorously shake for 15 seconds.
- 3 Allow to sit undisturbed for 15 minutes.
- 4 Observe for the presence of a continuous line of bubbles around the outside edge.

## PROCEDURE

## Procedure for Foam Stability Index

- 1 Add 0.5 mL of amniotic fluid to tubes containing increasing amounts of 95% ethanol ranging from 0.42–0.55 mL in 0.01-mL increments.
- 2 Vigorously shake for 15 seconds.
- 3 Allow to sit undisturbed for 15 minutes.
- 4 Observe for the presence of a continuous line of bubbles around the outside edge.
- 5 Values >47 indicate fetal lung maturity.

## MICROVISCOSITY

The presence of phospholipids decreases the microviscosity of the amniotic fluid. This change in microviscosity can be measured using the principle of fluorescence polarization employed by the Abbott TDx analyzer (Abbott Laboratories, Abbott Park, IL). The instrument measures the polarization of a fluorescent dye that combines with both surfactants and albumin. Dye bound to surfactant exhibits low polarization, whereas dye bound to albumin has high polarization. Albumin is used as an internal standard in the same manner as sphingomyelin because it remains at a constant level throughout gestation. The recorded changes in polarization produce a surfactant/albumin ratio expressed in milligrams surfactant to grams albumin that is compared with a standard curve that includes phosphatidyl glycerol and ranges from 0 to 160 mg/g (Figure 14–4). A ratio of 70 or greater provides a conservative prediction of FLM and lower values may be considered.<sup>2, 14</sup> Fluid should be filtered rather than centrifuged prior to examination to prevent sedimentation of the lipids, and use of contaminated fluid is not recommended.

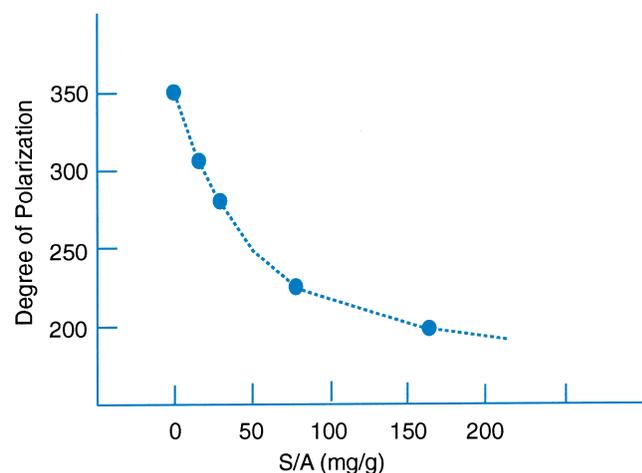


FIGURE 14–4 Sample TDx FLM calibration curve. (Adapted from Ashwood, et al.<sup>1</sup>)

## LAMELLAR BODIES AND OPTICAL DENSITY

The surfactants responsible for FLM are produced and secreted by the type II pneumocytes of the fetal lung in the form of structures termed lamellar bodies. The lamellar bodies enter the alveolar spaces to provide surfactant and also enter the amniotic fluid. Therefore, the number of lamellar bodies present in the amniotic fluid correlates with the amount of phospholipid present in the fetal lungs.

The presence of lamellar bodies increases the OD of the amniotic fluid. Specimens are centrifuged at 2000 g for 10 minutes and examined using a wavelength of 650 nm, which rules out interference from hemoglobin but not other contaminants, such as meconium. An OD of 0.150 has been shown to correlate well with an L/S ratio of greater than or equal to 2.0 and the presence of phosphatidyl glycerol.<sup>12</sup>

Lamellar bodies can be counted using resistance-pulse counting, such as that employed by Coulter cell-counting instruments (Beckman Coulter, Inc., Fullerton, CA.). Ranging in size from 1.7 to 7.3 fL, lamellar bodies can be counted using the platelet channel.<sup>1</sup> This technique is easily performed; however, samples must be free of particle contamination such as meconium and blood. A count of 32,000 or more particles per microliter represents adequate FLM.<sup>6</sup>

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## STUDY QUESTIONS

1. State two functions of amniotic fluid.
2. What is the primary cause of the normal increase in amniotic fluid as a pregnancy progresses?
3. State reasons for increased and decreased amounts of amniotic fluid.
4. Why might a creatinine level be requested on an amniotic fluid?
5. What is the purpose of placing a specimen of amniotic fluid in an amber-colored tube prior to sending it to the laboratory?
6. How are specimens for FLM testing delivered to and stored in the laboratory?
7. Why are specimens for cytogenetic analysis incubated prior to analysis?
8. What is the first processing procedure that the laboratory performs on amniotic fluid?
9. State the significance of the following colors in amniotic fluid: dark green, colorless, yellow, and red-brown.
10. What is the significance of a rise in the OD of amniotic fluid at 450 nm? 410 nm?
11. What is the purpose of plotting the amniotic fluid  $\Delta A_{450}$  on a Liley graph?
12. Why do neural tube disorders such as spina bifida and anencephaly produce increased amniotic fluid AFP?
13. Define MoM.
14. Explain the relationship between evaluation of amniotic fluid for HDN and FLM.
15. What is the role of lecithin in FLM?
16. Prior to 35 weeks' gestation, what is the normal L/S ratio? Why?
17. State two advantages of the Aminostat-FLM over the L/S ratio.
18. Does the failure to produce bubbles in the Foam Stability Index indicate increased or decreased lecithin? Why?
19. Relate the principle of the microviscosity test to the principle of the L/S ratio.
20. Does an L/S ratio of 2.0 correlate with a surfactant/albumin ratio of 40 mg/g? Why or why not?

21. What is the relationship of lamellar bodies and fluid OD at 650 nm to FLM?
22. Which test for FLM is least affected by contamination with hemoglobin and meconium? Why?

## CASE STUDIES AND CLINICAL SITUATIONS

1. Amniocentesis is performed on a woman believed to be in approximately the 31st week of gestation. This is the second pregnancy for this Rh-negative, woman with diabetes. Spectrophotometric analysis of the fluid shows a  $\Delta A_{450}$  of 0.3.
  - a. Based on the Liley graph, should the physician consider inducing labor?
  - b. What else must the physician consider prior to inducing labor?

The physician decides to induce labor based on a positive Aminostat-FLM.

  - c. What information did this test provide for the physician?
  - d. Why did the physician prefer an Aminostat-FLM over an L/S ratio in this situation?
2. Amniocentesis is performed following a maternal serum AFP level of 2.2 MoM at 15 weeks' gestation.
  - a. What fetal condition is suspected?
  - b. If the amniotic fluid AFP is 2.5 MoM, what additional test could be performed?
  - c. In what situation would this additional test not be performed?
3. If the degree of fluorescence polarization in an amniotic fluid is decreased, does this represent increased or decreased lecithin?
4. Amniotic fluid for FLM testing is centrifuged for 10 minutes at 5000 g. How will this affect the test results?
5. How might a dark green amniotic fluid affect the results of the following tests?
  - a. Foam Stability Index
  - b. L/S ratio
  - c. Aminostat-FLM
  - d.  $OD_{650}$
6. How might a blood-streaked amniotic fluid affect the results of the following tests?
  - a. L/S ratio
  - b. AChE
  - c. Bilirubin analysis
  - d. Aminostat-FLM

