1. The specimen of choice is 3-4 mL of amniotic fluid obtained by

2. Any scanning densitometer capable of accurately scanning the phospholipid bands at 525 nm may be used. The Helena EDC or the ClinScan 2 are recommended.

3. The Helena Fetal-Tek 200 is a semi-quantitative method used to aid in the estimation of fetal lung maturity by determining the lecithin to sphingomyelin (L/S) ratio of fetal amniotic fluid. This modification may be used for assessment of the presence of other phospholipids.

4. REAGENTS

5. Fetal-Tek 200 TLC Plate is a channelled thin layer chromatography plate consisting of two different surface areas: (a) a precipitin 3 mm across the lower edge of the plate and (b) a silica gel analytical layer comprising the remainder of the plate surface.

6. Preparation for Use: The Fetal-Tek 200 TLC Plate is ready for sampling. Do not package or package. Handling plates by edges only.

7. Storage and Stability: The plates should be stored in a dry, clean area at 15 to 30 °C. Do not store the plates in a warm oven or in a desiccator.

8. Sources of Error

9. Incomplete drying of the plate, before spraying with cupric acetate,

10. The reagent should be stored tightly closed...

11. WARNING: FOR IN-VITRO DIAGNOSTIC USE.

12. Ingredients:

13. Cupric Acetate Reagent (Cat. No. 8000)

14. Micro-Hood

15. Micro-Hood

16. Fetal-Tek 200 TLC Plates (10 x 20 cm) 8028

17. Fetal-Tek 200 Wicks (14 x 20 cm) 8023

18. Fetal-Tek 200 Marker (1 x 2 mL) 8026

19. Fetal-Tek Controls (10 x 5 mL each 8033

20. Fetal-Tek Controls (10 x 5 mL each 8033

21. The L/S ratio of 1.5 or less indicates immaturity L/S ratio of 1.5 to 1.9 indicates a transitional condition L/S ratio of 2.0 to 2.5 or greater indicates maturity.

22. The Helena Fetal-Tek 200 is a semi-quantitative method used to aid in the estimation of fetal lung maturity by determining the lecithin to sphingomyelin (L/S) ratio of fetal amniotic fluid. This modification may be used for assessment of the presence of other phospholipids.

23. SUMMARY

24. The presence of particulate matter in the solution may indicate product deterioration.

25. The marker should be a clear, colorless

26. The plates should be stored in a clean, dry area at 15 to 30 °C. Do not store the plates in a warm oven or in a desiccator.

27. Storage and Stability: The plates should be stored in a dry, clean area at 15 to 30 °C. Do not store the plates in a warm oven or in a desiccator.

28. Signs of Deterioration: The plates should not be used if they are wet or scratched.

29. HARMFUL.

30. WARNING: FOR IN-VITRO DIAGNOSTIC USE.

31. Ingredients:

32. Cupric Acetate Reagent contains 3% (w/w) cupric acetate in 8% (v/v) phosphoric acid.

33. WARNING: FOR IN-VITRO DIAGNOSTIC USE.

34. Preparation for Use: The Fetal-Tek 200 TLC Plate is ready for sampling. Do not package or package. Handling plates by edges only.

35. Storage and Stability: The plates should be stored in a dry, clean area at 15 to 30 °C. Do not store the plates in a warm oven or in a desiccator.

36. Signs of Deterioration: The plates should not be used if they are wet or scratched.

37. WARNING: FOR IN-VITRO DIAGNOSTIC USE.

38. Preparation for Use: The Fetal-Tek 200 Marker is a channeled thin layer chromatography panel consisting of two different surface areas: (a) a precipitin 3 mm across the lower edge of the plate and (b) a silica gel analytical layer comprising the remainder of the plate surface.

39. Storage and Stability: The plates should be stored in a dry, clean area at 15 to 30 °C. Do not store the plates in a warm oven or in a desiccator.

40. Signs of Deterioration: The plates should not be used if they are wet or scratched.

41. WARNING: FOR IN-VITRO DIAGNOSTIC USE.

42. Preparation for Use: The Fetal-Tek 200 Marker is a channeled thin layer chromatography panel consisting of two different surface areas: (a) a precipitin 3 mm across the lower edge of the plate and (b) a silica gel analytical layer comprising the remainder of the plate surface.

43. Storage and Stability: The plates should be stored in a dry, clean area at 15 to 30 °C. Do not store the plates in a warm oven or in a desiccator.

44. Signs of Deterioration: The plates should not be used if they are wet or scratched.

45. WARNING: FOR IN-VITRO DIAGNOSTIC USE.

46. Preparation for Use: The Fetal-Tek 200 Marker is a channeled thin layer chromatography panel consisting of two different surface areas: (a) a precipitin 3 mm across the lower edge of the plate and (b) a silica gel analytical layer comprising the remainder of the plate surface.

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48. Signs of Deterioration: The plates should not be used if they are wet or scratched.

49. WARNING: FOR IN-VITRO DIAGNOSTIC USE.

50. Preparation for Use: The Fetal-Tek 200 Marker is a channeled thin layer chromatography panel consisting of two different surface areas: (a) a precipitin 3 mm across the lower edge of the plate and (b) a silica gel analytical layer comprising the remainder of the plate surface.

51. Storage and Stability: The plates should be stored in a dry, clean area at 15 to 30 °C. Do not store the plates in a warm oven or in a desiccator.

52. Signs of Deterioration: The plates should not be used if they are wet or scratched.

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54. Preparation for Use: The Fetal-Tek 200 Marker is a channeled thin layer chromatography panel consisting of two different surface areas: (a) a precipitin 3 mm across the lower edge of the plate and (b) a silica gel analytical layer comprising the remainder of the plate surface.

55. Storage and Stability: The plates should be stored in a dry, clean area at 15 to 30 °C. Do not store the plates in a warm oven or in a desiccator.

56. Signs of Deterioration: The plates should not be used if they are wet or scratched.

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58. Preparation for Use: The Fetal-Tek 200 Marker is a channeled thin layer chromatography panel consisting of two different surface areas: (a) a precipitin 3 mm across the lower edge of the plate and (b) a silica gel analytical layer comprising the remainder of the plate surface.

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64. Signs of Deterioration: The plates should not be used if they are wet or scratched.

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66. Preparation for Use: The Fetal-Tek 200 Marker is a channeled thin layer chromatography panel consisting of two different surface areas: (a) a precipitin 3 mm across the lower edge of the plate and (b) a silica gel analytical layer comprising the remainder of the plate surface.

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68. Signs of Deterioration: The plates should not be used if they are wet or scratched.

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70. Preparation for Use: The Fetal-Tek 200 Marker is a channeled thin layer chromatography panel consisting of two different surface areas: (a) a precipitin 3 mm across the lower edge of the plate and (b) a silica gel analytical layer comprising the remainder of the plate surface.

71. Storage and Stability: The plates should be stored in a dry, clean area at 15 to 30 °C. Do not store the plates in a warm oven or in a desiccator.

72. Signs of Deterioration: The plates should not be used if they are wet or scratched.

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74. Preparation for Use: The Fetal-Tek 200 Marker is a channeled thin layer chromatography panel consisting of two different surface areas: (a) a precipitin 3 mm across the lower edge of the plate and (b) a silica gel analytical layer comprising the remainder of the plate surface.

75. Storage and Stability: The plates should be stored in a dry, clean area at 15 to 30 °C. Do not store the plates in a warm oven or in a desiccator.

76. Signs of Deterioration: The plates should not be used if they are wet or scratched.

77. WARNING: FOR IN-VITRO DIAGNOSTIC USE.

78. Preparation for Use: The Fetal-Tek 200 Marker is a channeled thin layer chromatography panel consisting of two different surface areas: (a) a precipitin 3 mm across the lower edge of the plate and (b) a silica gel analytical layer comprising the remainder of the plate surface.

79. Storage and Stability: The plates should be stored in a dry, clean area at 15 to 30 °C. Do not store the plates in a warm oven or in a desiccator.

80. Signs of Deterioration: The plates should not be used if they are wet or scratched.
ammoniaca31c. 12
2. Vaginally obtained samples are satisfactory only if care is taken to obtain a “clean” sample. It should only be used if the fluid is freely flowing and carefully tapped. 12
Interfering Factors
1. Excessive speed or prolonged centrifugation may sediment phospholipids. 12 Incomplete charring or charring at too high a temperature may also cause inaccurate results.
2. Filtration of the fluid with some materials may cause falsely lower results. Some filter papers may absorb up to 90% of the lecithin. 12
3. Chronic acid should not be used to clean any glassware. Traces of chronic acid may hydrolyze the phospholipids. 12
4. Vaginal samples are easily contaminated with mucus and bacteria that contain phospholipase A. These should only be used with caution. 12
5. McEwen present in the sample may interfere with phase separation of the lipid extraction step and cause erroneous results. 12
6. The L/S ratio obtained on amniotic fluid contaminated with blood will probably be inaccurate because blood contains lecithin and sphingomyelin. 12
Preparation and Storage Immediately after collection, the specimen should be centrifuged at slow speed (1000 RPM or about 500 x g) for 3 minutes and the supernatant removed for use in this procedure. No special additives or preservatives are required. If storage of the specimen is necessary, it should be frozen below -30 °C to avoid repeated freezing and thawing. If the specimen is left at room temperature for any length of time, any phospholipase present may destroy the phospholipids.

PROCEDURE
Materials Provided: The following materials needed for the Helena Fetal-Tek 200 Method are provided in the Fetal-Tek 200 Equipment Kit (Cat. No. 8022). Individual items are also available.

Hardware
- Fetal-Tek 200 Developing Chamber
- Micro-Hood
- Micropreparation Dish
- Microdispenser and Tubes (50 µl)
- Micro-Hood over the Micropreparation Dish
- Centrifuge
- Centrifuge Tube
- Microcentrifuge
- Erlenmeyer Flask
- 9” Pasteur pipette
- Pipetman
- Blender
- Vortex mixer
- Transfer pipettes (Pasteur type, 9"")

Chemicals
- 18°C (56°F) Oven
- Deionized Water
- Methanol 90%
- Chloroform 30 mL
- 2-Propanol 25.0 mL
- Triethylamine 25.0 mL
- Solvent: Chloroform 30 mL, Methanol 9.0 mL, 2-Propanol 25.0 mL, Triethylamine 25.0 mL, Deionized Water 7.0 mL

Materials Required but not Provided:
- Triethylamine Reagent
- Chloroform (reagent grade)
- 2-propanol (reagent grade)
- Glass screw cap culture tubes or test tubes (16 x 125 mm) for extraction phase
- Vortex mixer
- Transfer pipettes (Pasteur type, 9"")
- Centrifuge
- Erlenmeyer Flask

STEP BY STEP METHOD
NOTE: All steps of the procedure involving the solvent must be performed under a hood.

A. Preparation of the Fetal-Tek 200 Developing Chamber (Mobile Preparation)

1. Prepare the solvent for the developing chamber by adding the ingredients to an Erlenmeyer flask in the order listed. Place the flask on a magnetic stirrer for 20 minutes. If a magnetic stirrer is not available, mix the reagents well by swirling the flask after the addition of each reagent. Proper mixing of the water is especially critical. The water must be the last ingredient added and must be added very slowly with constant mixing. Improper mixing results in a cloudy solution indicating that the water has not mixed with the other reagents as it should. The final mixture should be a clear, colorless solution.
2. For 2 plates, use:
   - Chloroform 30 mL
   - Methanol 9.0 mL
   - 2-Propanol 25.0 mL
   - Triethylamine 25.0 mL
3. Determine the lecithin/sphingomyelin ratio according to instructions in RESULTS. Calculation of the Unknown. NOTE: Although the Fetal-Tek 200 Plate is larger than other plates you may be accustomed to using, it will fit into most densitometers. Should the plate not fit into your densitometer, you can easily cut it to proper size using a small glass cutter. Cut the plate in a circular motion, keeping the cut side of the plate away from the densitometer.

B. Development of the Fetal-Tek 200 Plate

1. Spray the plate with Cupric Acetate Reagent until the entire plate is completely wet.
2. Place the plate on a blotter pad in a 180° angle.
3. Pour 40 mL of solvent onto the wick, saturating as much of it as possible. The solvent should penetrate the entire chamber at least 1 cm from the bottom. Allow the samples to evaporate to dryness (approximately 30 minutes).
4. Add 2 mL of chloroform into each tube. Mix by vortexing for 10-15 seconds.
5. Add 2 mL chloroform into each tube. Mix by vortexing for 20-25 minutes.
6. Centrifuge the tubes at high speed (1500 RPM or about 1500 x g for at least 10 minutes or until the samples are completely separated. There will be three distinct phases: a top methanol layer, a center protein “button”, and a lower chloroform layer.
7. Remove no more than 20 µl of the chloroform layer (bottom layer), taking care not to aspirate any of the methanol or protein layer.
8. Transfer the chloroform layer to a well in the Micropreparation Dish. Discard the pipette.
9. Repeat Step 8-6 for all samples and controls until all the chloroform extracts have been transferred to the Micropreparation Dish. Use a clean Pasteur pipette for each extraction.
10. Place the plate in the Fetal-Tek 200 Developing Chamber with the preadsorbent layer at the bottom and the silica gel area facing the Wick. Figure 1: Fetal-Tek 200 Plate showing the location of the phospholipid bands.
3. Using the 50 µL Microdispenser, apply 40 µL of the Fetal-Tek 200 Marker as a streak across the appropriate channel.

4. Add 40 µL of chloroform to the first evaporated sample in the Micropreparation Dish. Swirl 10 seconds to completely dissolve the extracted phospholipids.

5. Streak the entire amount of reconstituted sample across one channel of the plate using the 50 µL Microdispenser. Discard the tip on the Microdispenser after each sample application.

6. Repeat Steps 4 and 5 until all samples and controls have been streaked on appropriate channels of the plate.

7. Place the plate under the Micro-Hood with only the fan on (no heat). Allow the applications to dry completely (approximately 4 minutes).

8. Place the plate in the Fetal-Tek 200 Developing Chamber with the adsorbent layer at the bottom and the silica gel area facing the wick.

9. Allow the phospholipids to migrate for approximately 70 minutes.

10. Remove the plate from the developing chamber and dry in a 180°C (356°F) oven with forced air for 2-3 minutes.

D. DEVELOPMENT OF THE PHOSPHOLIPID BANDS

1. Spray the plate with Cupric Acetate Reagent until the entire plate is completely wet.

2. Place the plate on a blotter pad in a 180°C oven. Allow the bands to char until they show a maximum bluish-grey color (approximately 7-10 minutes). See RESULTS, Sources of Error, No. 2 for effects of improper charring.

3. Identify the phospholipids present in the test sample by comparison to the Fetal-Tek 200 Marker.

4. Scan the charred lecithin and sphingomyelin bands in a scanning densitometer with a 525 nm filter.

5. Determine the lecithin/sphingomyelin ratio according to instructions in RESULTS. Calculation of the Unknown.

NOTE: Although the Fetal-Tek 200 Plate is larger than other plates you may be accustomed to using, it will fit into most densitometers. Should the plate not fit into your densitometer, you can easily cut it to proper size using a small glass cutter. Cut the plate only after completing the procedure. Place the plate on a clean sheet of paper, silica side down, to protect the silica. Using a ruler as a guide, draw the glass cutter across the width of the plate making a good scribe line so the plate will break evenly. If you wish to preserve all the bands you may have to make two cuts—one above and one below the pattern. As a standard guide, cuts should be made 11.5 cm and 2 cm from the bottom of the plate. If preserving the PG band is not important, one cut may be made 9 cm from the bottom of the plate.

STABILITY OF FINAL REACTION PRODUCT: All determinations made on the Fetal-Tek 200 TLC Plate should be made within 4 hours after charring. The bands have a tendency to fade during storage.

CALIBRATION: A calibration curve is not necessary because relative concentration of sphingomyelin to lecithin is the only parameter measured.

QUALITY CONTROL: The Fetal-Tek 200 Marker and the three levels of Fetal-Tek Controls (Immature, Borderline, and Mature) should be run on each Fetal-Tek 200 TLC Plate to verify all aspects of the procedure.

1. Fetal-Tek 200 Marker: This marker is used to show the location of the lecithin, sphingomyelin, PS, PI, and PE on the plate. Inadquate or irregular separation of the marker indicates improper technique in the sample application or migration phase of the procedure. Do not quantitate the marker.

2. L/S Ratios Controls (Immature, Borderline, and Mature): The controls, when reconstituted as directed, should be treated in exactly the same manner as the amniotic fluid. They should be carried through all phases of the procedure concurrently with the patient samples. L/S ratios determined on the controls should fall within the ranges stated on the package insert provided with each lot of control. If controls' migration appears inadequate or irregular, as compared to Fetal-Tek 200 Marker, and they do not quantitate, the problem may be in the extraction phase. However, if controls do not quantitate correctly but migration appears acceptable, the most common causes are incomplete charring or poor densitometer linearity.

3. If results with both the Fetal-Tek 200 Marker and the controls are as expected, but the migration of the test samples appears poor, the sample itself may have been contaminated or otherwise improperly collected or handled.

RESULTS

The order of migration of the phospholipids from the application site is as follows: sphingomyelin, lecithin, PS, PI, PE, and neutral lipids. The phospholipids should be identified by comparison to the Fetal-Tek 200 Marker. Figure 1 illustrates the position of the phospholipids on the Fetal-Tek 200 TLC Plate.

**Figure 1:** Fetal-Tek 200 Plate showing the location of the phospholipid bands.
Beaumont, Texas

1. The specimen of choice is 3-4 mL of amniotic fluid obtained by

2. The preadsorbent area on the plate facilitates sample appli-

3. The presence of a "pseudo" PG band has been documented in some cases.

4. Improperly handled specimens are a common source of error. This

5. The lecithin and sphingomyelin bands do not develop at exactly the

6. The reagent should be stored tightly closed

7. The plates should not be used if they are

8. Considering the literature provides three important facts concerning PG: (1) in the majority of cases in which PG was

9. While research indicates that PI is an important surfactant component futher studies are needed to determine its exact role.


14. Applications

15. The L/S ratio has gained acceptance as the pregnancy index of fetal maturity. The critical values of L/S ratios as related to fetal lung maturity are divided into three cate-

16. The Fetal-Tek 200 TLC Plate is a channeled thin layer chromatography plate consisting of two different surface areas: (1) a preservative 3.0 mm across the lower edge of the plate and (2) a silica gel analytical layer comprising the remainder of the plate surface.

17. Storage and Stability: The plates should be stored in a dry, clean area at 15 °C to 30 °C. Do not store the plates in a warm oven or in a desiccator. The expiration date indicates the date beyond which the plate shall not be used.


19. Fetal-Tek 200 Marker is a chloroform solution, and is volatile. Do not breathe the fumes. Keep the vial tightly capped when not in use.


21. Signs of Deterioration: The plates shall not be used if they are wet or scratched.

22. Fetal-Tek 200 TLC Plate (Cat. No. 8528)

23. Sphingomyelin (bovine brain) .....................................0.03 mg/mL

24. Lecithin (egg yolk) .............................................0.07 mg/mL

25. Phosphatidylglycerol (egg yolk) ..................................0.15 mg/mL

26. For Sales, Technical and Order Information and Service Assistance, call 800-231-5663 toll free.

27. Helena Fetal-Tek 200 Kit

28. Sample Cat. No.

29. Hardware

30. Fetal-Tek 200 Developing Chamber

31. Micro-Hood

32. MicroPreparation Dish

33. Microdispenser and Tubercules (5 mL)

34. Consumables

35. Fetal-Tek 200 TLC Plates (10 x 20 cm)

36. Fetal-Tek 200 Wicks (14 x 20 cm)

37. Fetal-Tek Controls (10 x 5 mL each)

38. Cupric Acetate Reagent (3 x 250 mL)

39. Fetal-Tek TLC Spray C

40. Hardware Marker

41. Glue Stick

42. Glue Strips

43. Fetal-Tek 200 Report Forms (1 pad of 100)

44. BIBLIOGRAPHY

45. Helena Laboratories reserves the right to make product specifications and packaging changes from time to time without prior notice.