# Fetal Lung Maturity Testing: Current Challenges and Considerations

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# Disclosures

- David G. Grenache has no relevant conflicts of interest to disclose

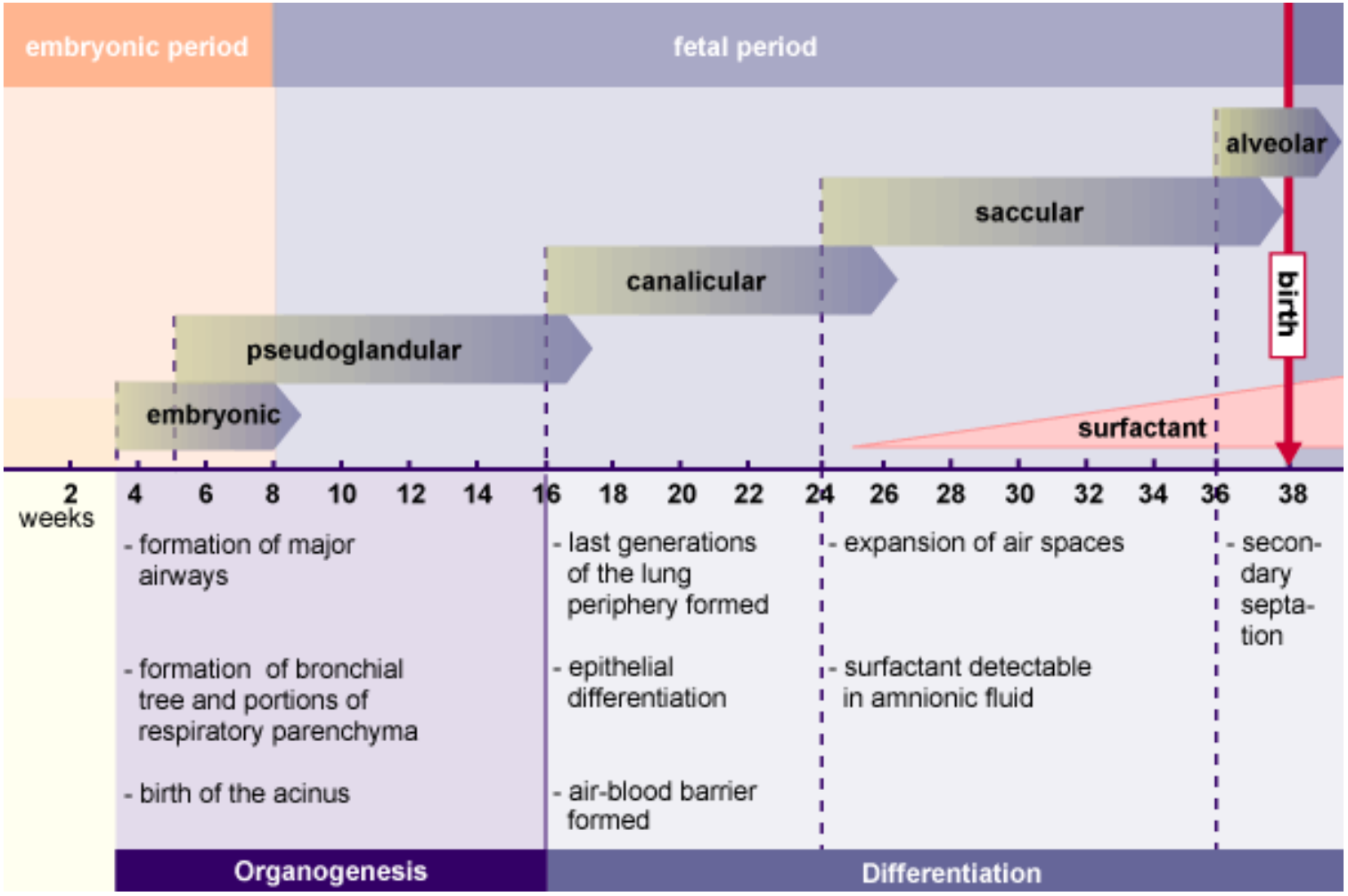
# Objectives

1. Describe the pathophysiology and treatment of newborn respiratory distress syndrome

2. Compare and contrast the different types of fetal lung maturity tests and discuss their clinical utility

3. Discuss contemporary issues facing fetal lung maturity testing

# Fetal Lung Development

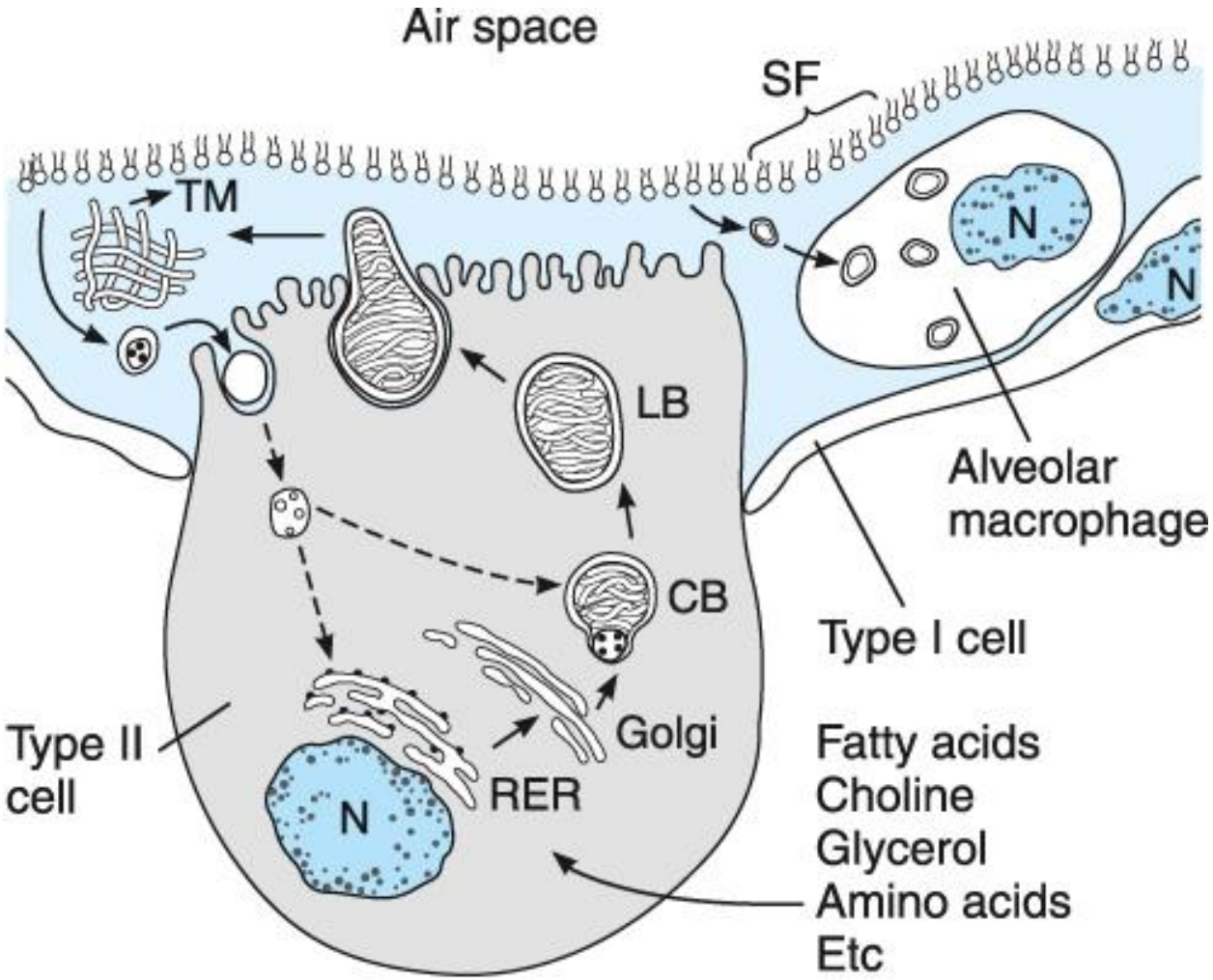



http://www.embryology.ch/anglais/rrespiratory/phasen01.html

# Surface Tension & Surfactant

- Internal surface of the alveolus is covered with a thin coating of fluid
- Water in this fluid has a high surface tension which promotes collapse the alveolus
- Pulmonary surfactants decrease surface tension of water
  - Increases lung compliance
  - Prevents collapse of alveoli during expiration

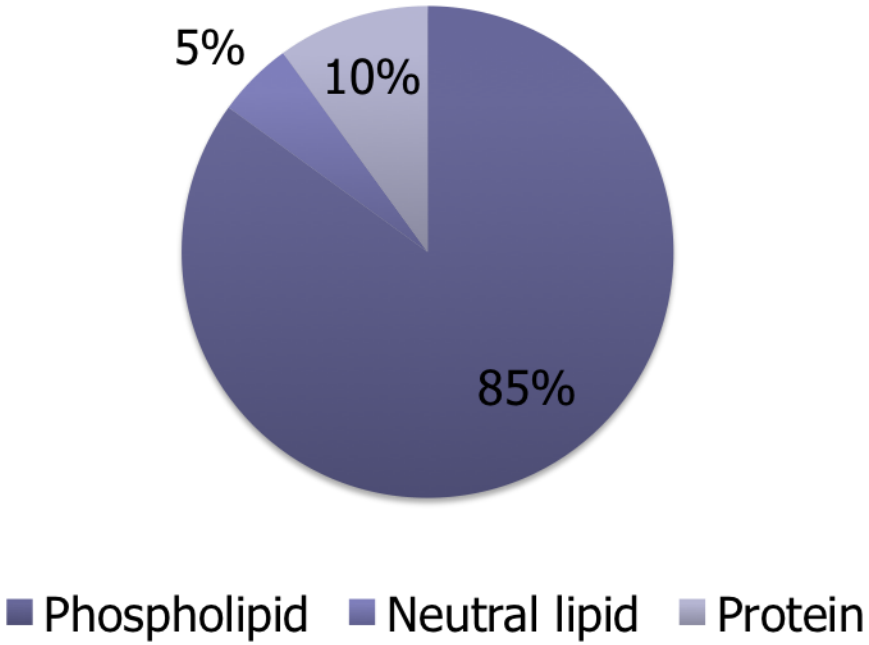
# Surfactant Metabolism



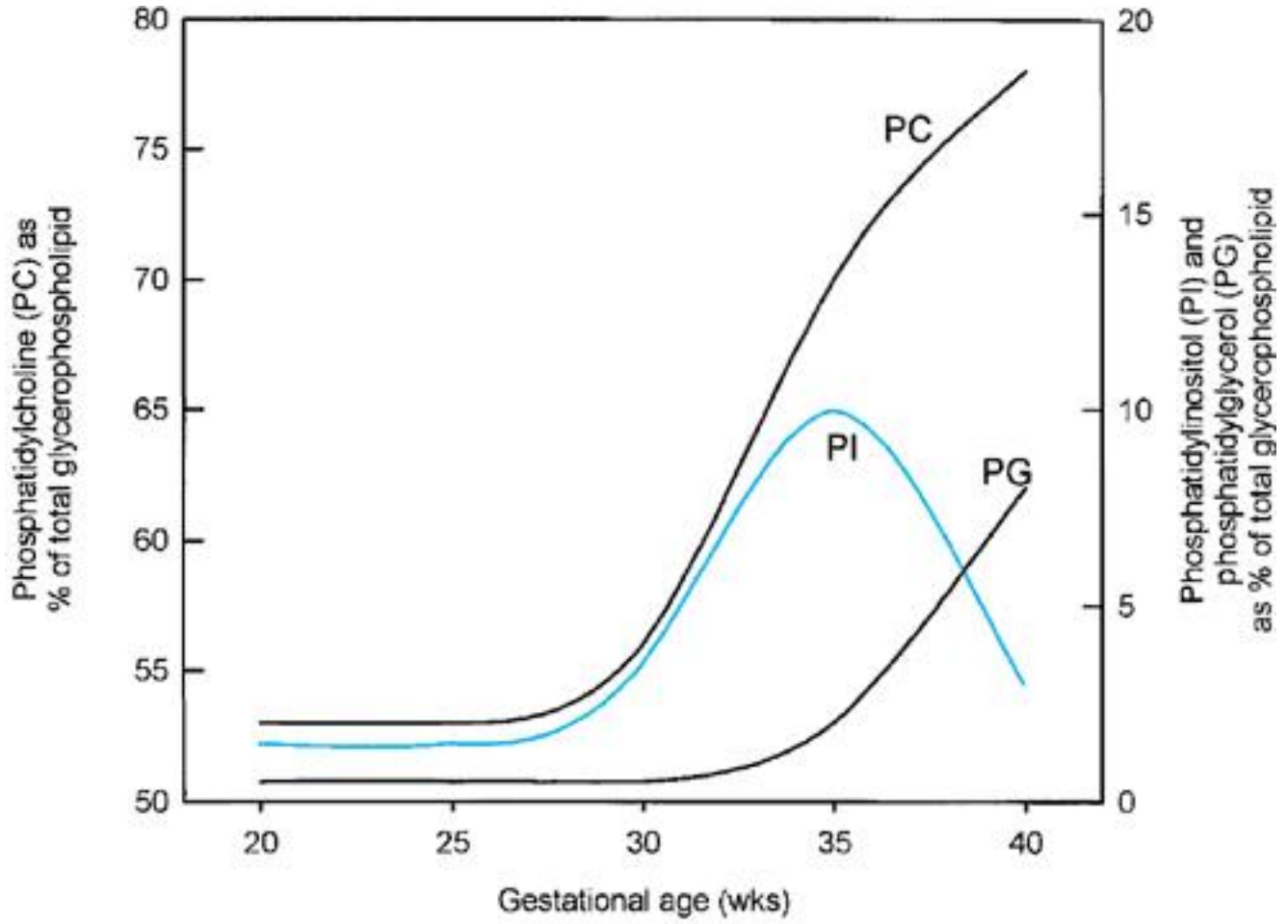

*Am Rev Respir Dis* 1987;136:426

# Pulmonary Surfactants

- Phosphatidylcholine (76%)
  - AKA lecithin
- Phosphatidylglycerol (13%)
- Phosphatidylinositol (4%)
- Phosphatidylethanolamine (3%)
- Sphingomyelin (2%)
- Other phospholipids (2%)



# Temporal Expression of Surfactant




*Williams Obstetrics*, 22nd edition

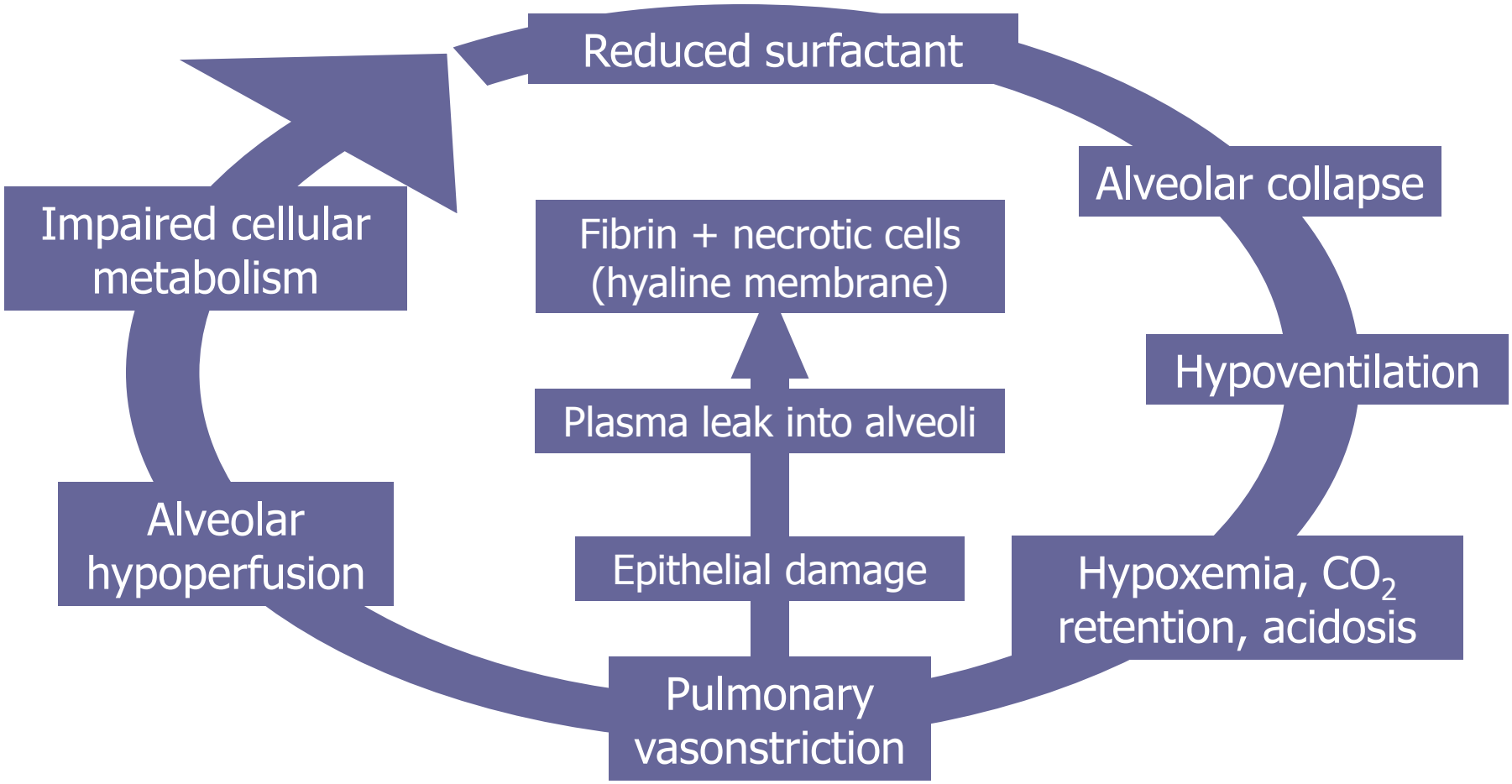
# Respiratory Distress Syndrome (RDS)

- Was known as hyaline membrane disease
- Caused by a deficiency in pulmonary surfactant
- Most common cause of respiratory failure in neonates
- Incidence is indirectly proportional to gestational age at delivery

| Weeks gestation at birth | Incidence (%) |
|---|---|
| <28 | 60-80 |
| 32-36 | 15-30 |
| >37 | 5 |
| Term | Rare |

~20,000 newborns/year (US)

# Pathophysiology of RDS



# RDS Risk Factors

- Prematurity (<37 weeks)
- Male sex
- Caucasian race
- Delivery by C-section
- 2nd born infant of twins
- History of RDS in sibling



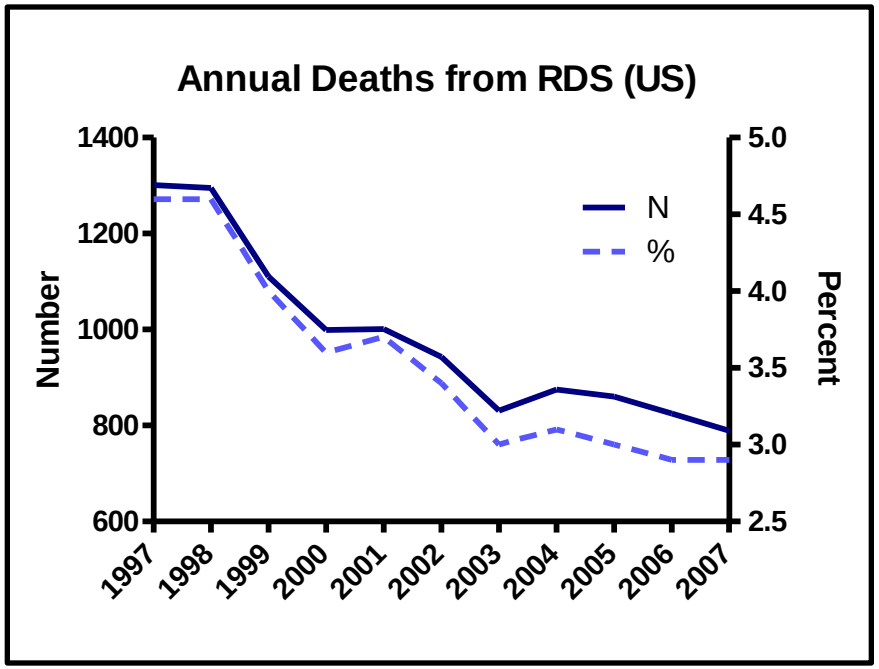
# Clinical Manifestations of RDS

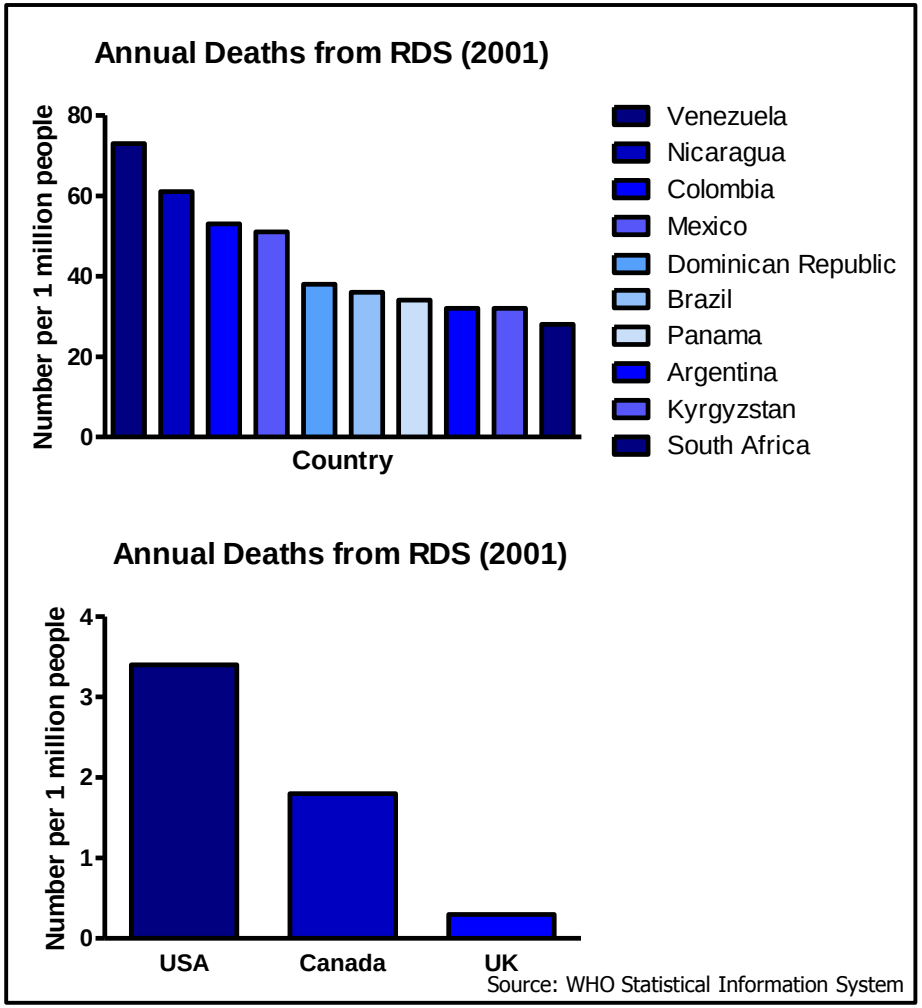
- Tachypnea
- Prominent, audible grunting
- Nasal flaring
- Subcostal and intercostal retractions
- Cyanosis unresponsive to oxygen administration
- Progressive worsening of cyanosis and dyspnea
- Apnea and irregular respirations

# Prevention/Treatment of RDS

- Antenatal prevention
  - Prevention of preterm delivery
  - Maternal administration of corticosteroids stimulates fetal synthesis of surfactant
- Surfactant replacement
  - Credited for largest drop in infant mortality in 25 years
  - Prophylaxis of preterm infants at risk for RDS
  - Treatment of infants with RDS
- Continuous positive airway pressure (CPAP) or assisted ventiliation
  - Keeps alveoli open at expiration

# RDS Statistics

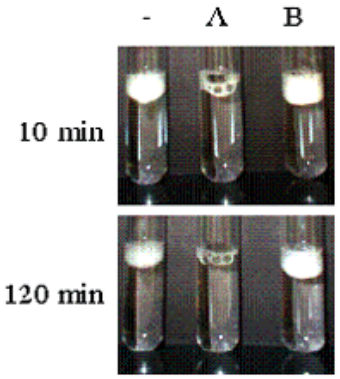


# Tests for Fetal Lung Maturity (FLM)

- Performed on amniotic fluid
  - 32-$38^{6/7}$ weeks of gestation (ACOG 2008)
- Used for decision making
  - Allow or delay delivery w/ steroid administration
  - Uncertain gestational age
  - Transfer mother to facility with NICU
- Must have high sensitivity for immaturity & high negative (mature) predictive value
- Performed rapidly (ideally)

# FLM Test History




Foam Stability

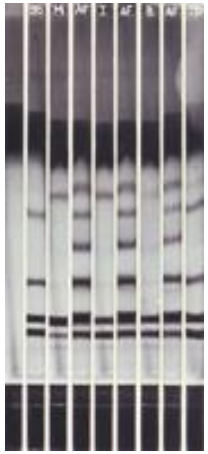



OD 650



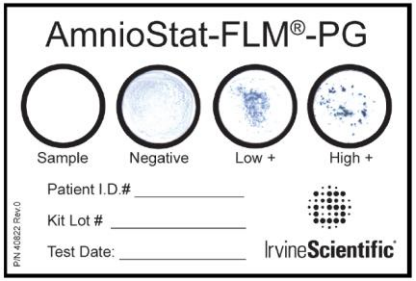
S/A Ratio

1971 1972 1979 1980 1983 1986 1988

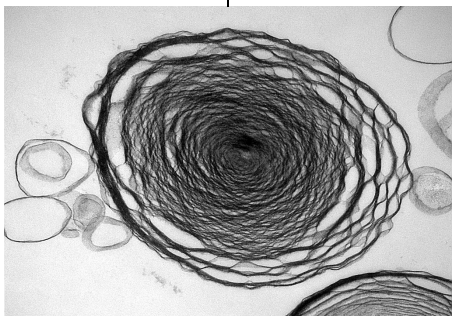


L/S Ratio

PG by TLC



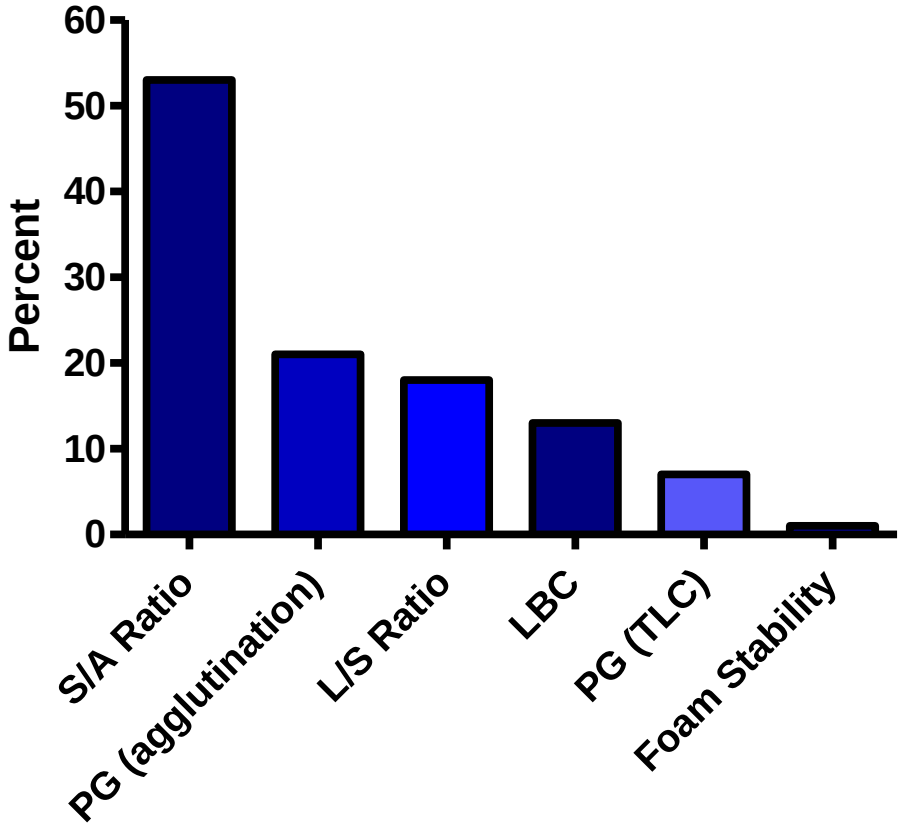

PG by agglutination



Lamellar Body Count

# FLM Tests Offered by U.S. Labs

Percent of total (N=251)

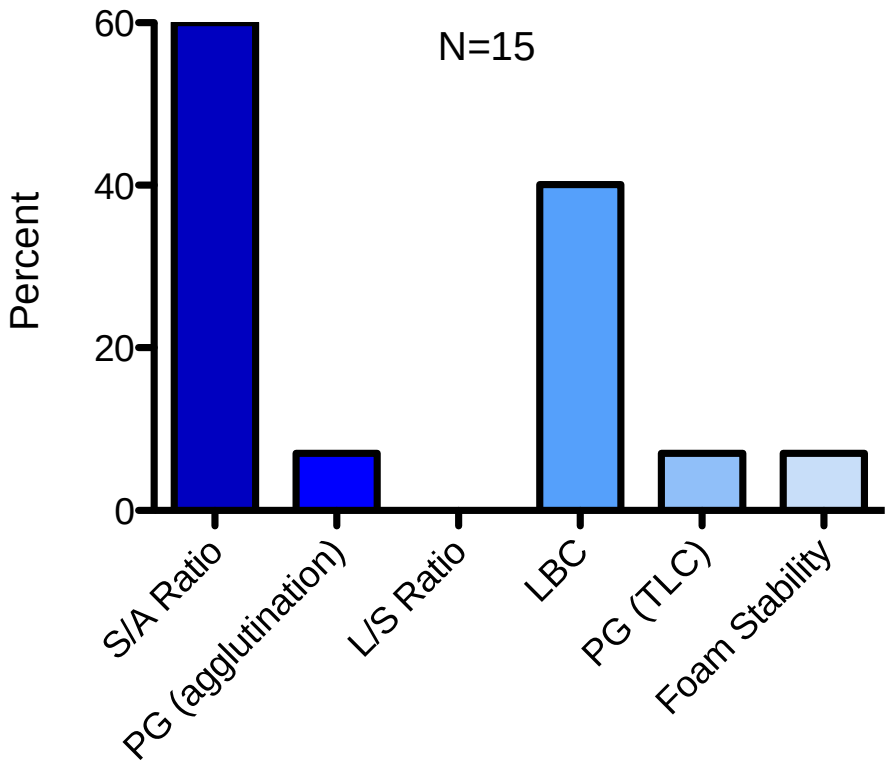



Grenache, et al. *Clin Chim Acta* 2010 411:1746-49

# FLM Tests Offered by Canadian Labs

Percent of total (N=30)

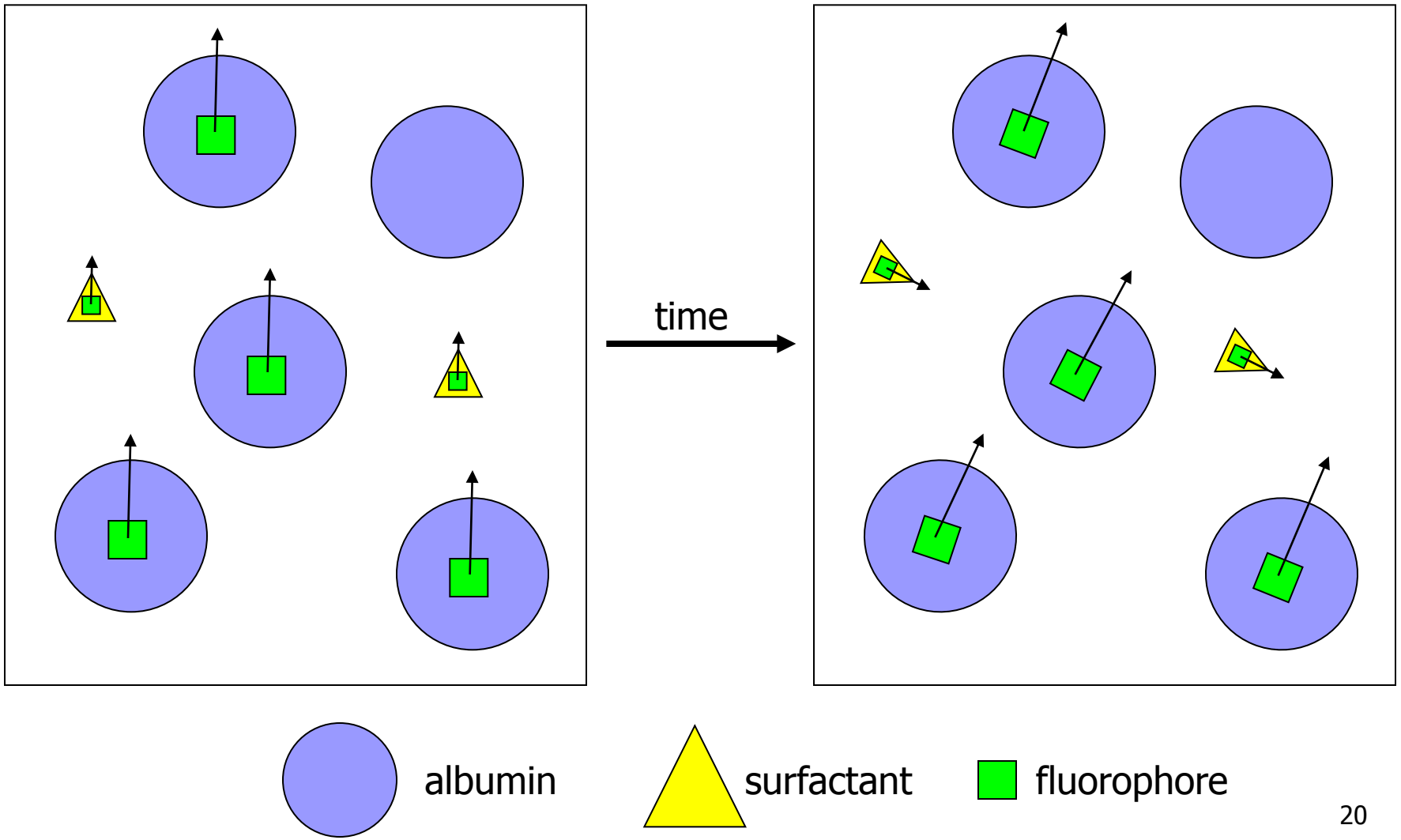
- 50% do not perform FLM tests
  - Adult and child patients only
  - Clinical necessity of test is questionable
  - Lack of clinical demand
  - Lack of EPT, cell counters are currently being replaced so creates difficulty with validation, lack of correlation with TDx
  - MDs do not ask for this test
  - No (infant) delivery in my hospital
  - No demand from OB
  - No obstetrics
  - Not relevant to our practice



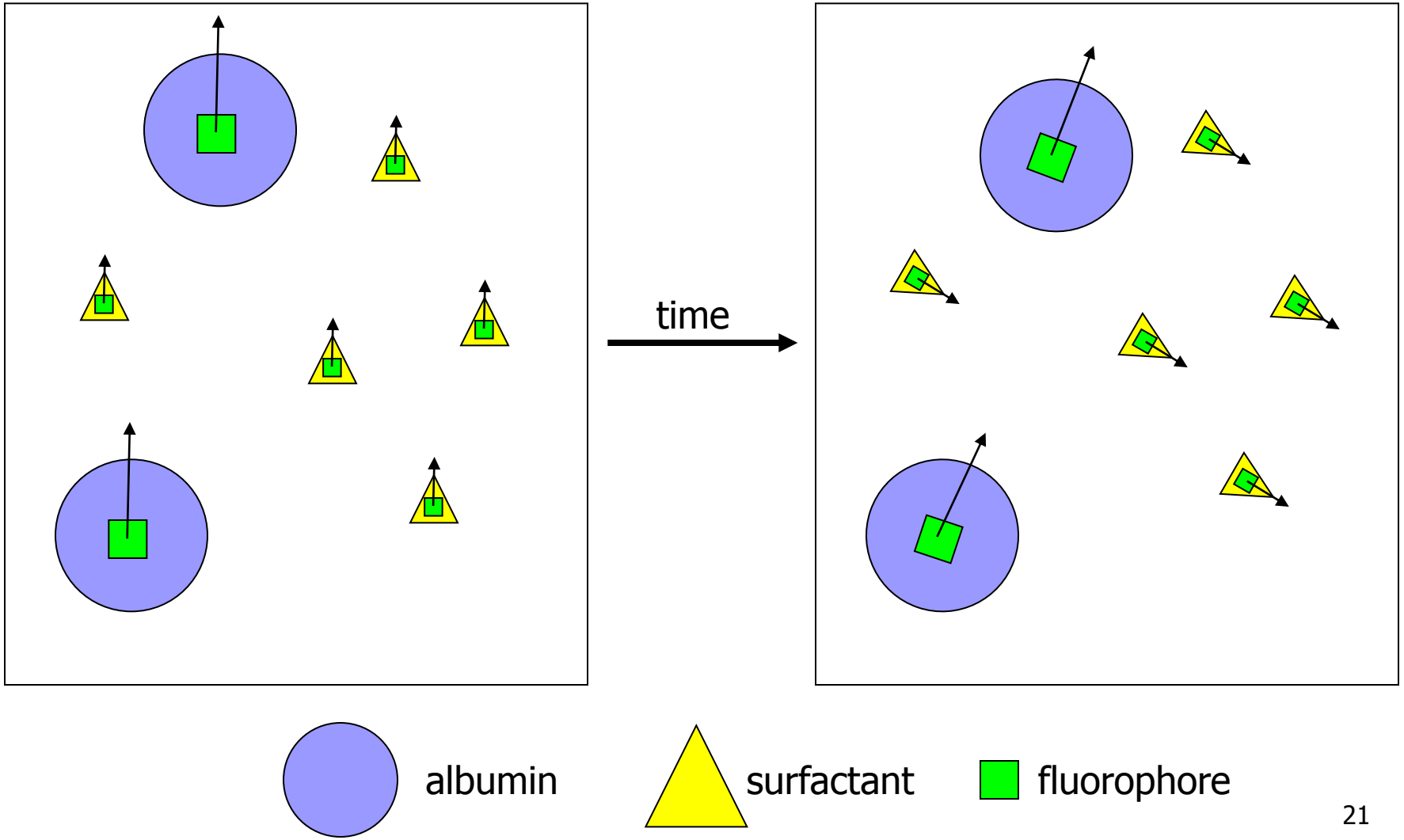
# Surfactant/Albumin (S/A) Ratio

- Fluorescence polarization
  - Decrease in light polarization due to partitioning of a fluorophore between surfactant and albumin
- Change in polarization is a function of the rate of molecular rotation of surfactant and albumin

# Low Surfactant: High Net Polarization



# High Surfactant: Low Net Polarization



# S/A Ratio

Advantages

- High sensitivity for immaturity
- Rapid and precise (CV ~5%)
- Quantitative
- Standardized
- QC/PT available

Disadvantages

- Effected by blood and meconium
- Wide grey zone
- Single vendor

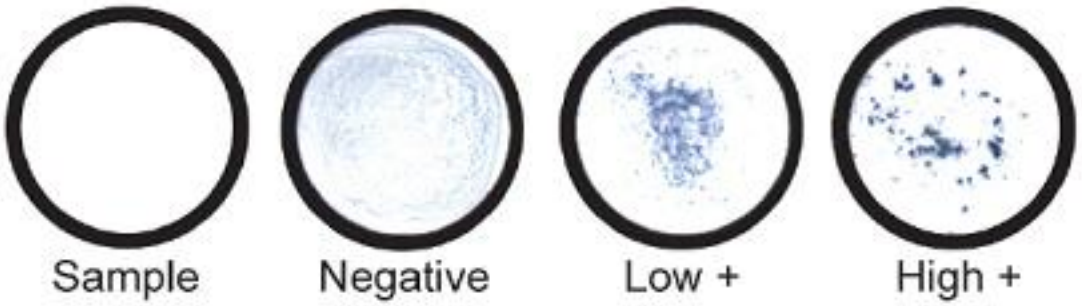
| | |
|---|---|
| Mature | $\geq$55 mg/g |
| Indeterminate | 40-54 mg/g |
| Immature | $\leq$39 mg/g |

# S/A Ratio Performance

| Reference | N | Cutoff (mg/g) | Sensitivity (%) | Specificity (%) | Immature PV (%) | Mature PV (%) |
|---|---|---|---|---|---|---|
| *Clin Chem* 1997 43:S196 | 50 | 40 | 100 | 60 | 14 | 100 |
| *Clin Chem* 1998 44:A157 | 94 | 55 | 100 | 71 | 29 | 100 |
| *Clin Chem* 2002 48:761 | 185 | 55 | 100 | 72 | 24 | 100 |
| *Clin Chem* 2002 48:761 | 185 | 45 | 100 | 84 | 36 | 100 |
| *Clin Chim Acta* 2002 326:61 | 303 | 55 | 96 | 70 | 36 | 99 |

# PG by Agglutination

- Qualitative agglutination test
  - PG is incorporated into lipid particles
  - Anti-PG antibodies induce agglutination



# PG by Agglutination

Advantages

- High sensitivity for immaturity
- Quick & simple to perform
- Unaffected by blood or meconium
- QC/PT available

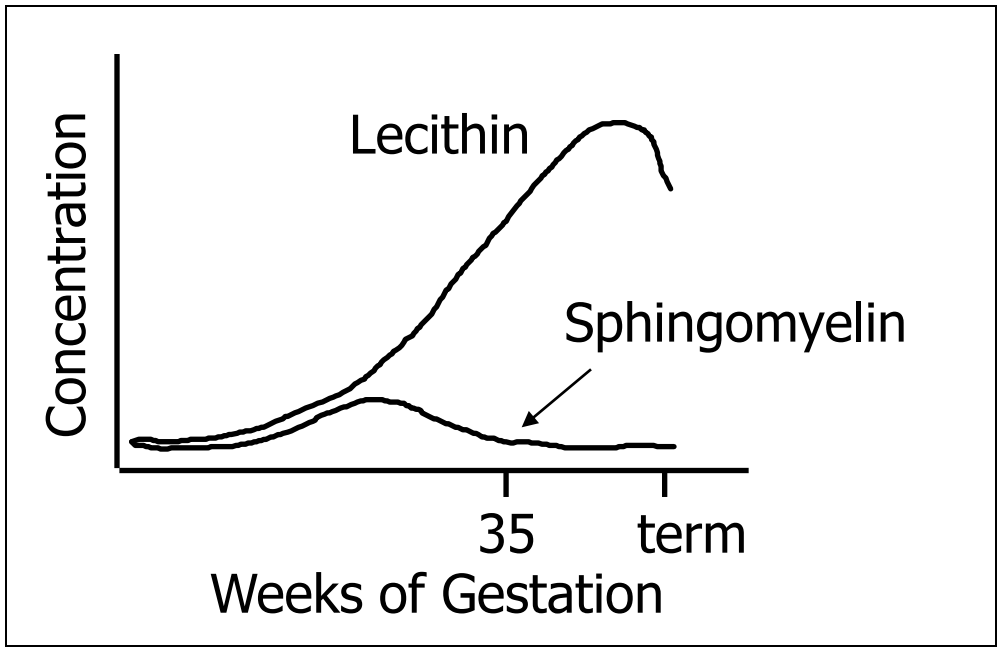
Disadvantages

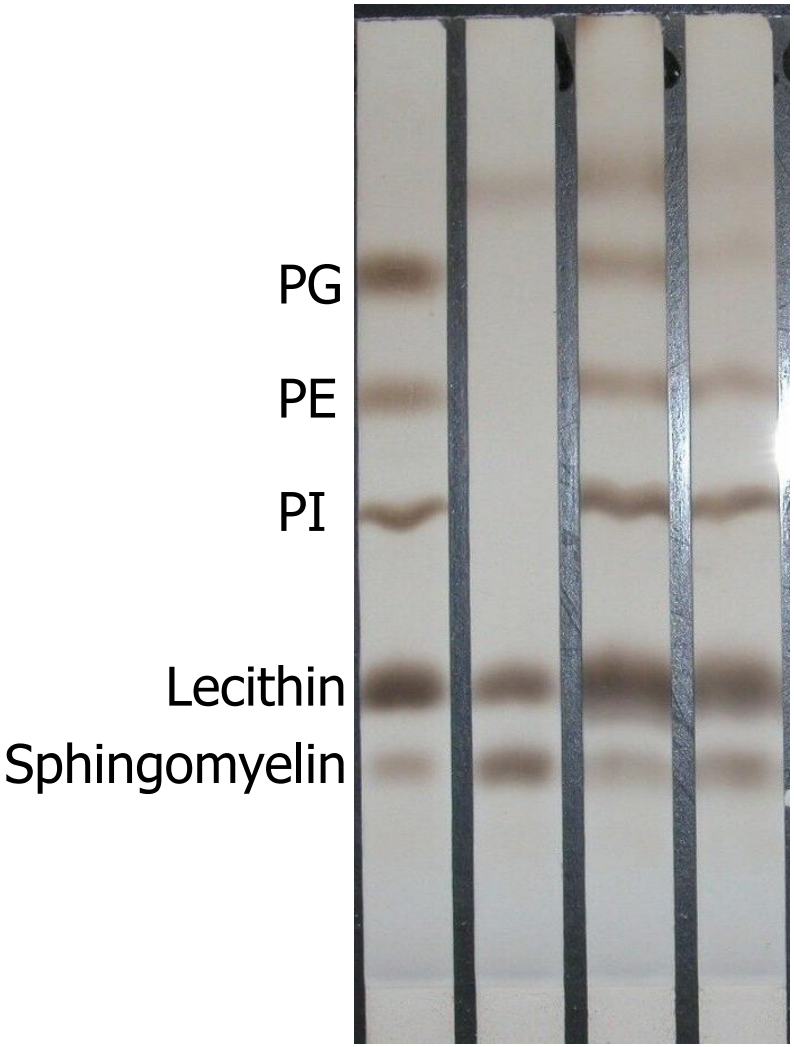
- PG is last surfactant to increase
- Subjective test interpretation
- Single vendor

# PG Performance

| Reference | N | Sensitivity (%) | Specificity (%) | Immature PV (%) | Mature PV (%) |
|---|---|---|---|---|---|
| *AJOG* 1983 147:681 | 74 | 100 | 80 | 38 | 100 |
| *Obstet Gynecol* 1984 63:52 | 150 | 92 | 73 | 23 | 99 |
| *Clin Chem* 1984 30:1233 | 49 | 100 | 68 | 39 | 100 |
| *Am J Perinatol* 1985 2:88 | 91 | 100 | 48 | 21 | 100 |
| *AJOG* 1985 151:1061 | 119 | 100 | 88 | 26 | 100 |
| *AJCP* 1989 1:293 | 40 | 83 | 85 | 50 | 97 |
| *AJOG* 1989 160:298 | 67 | 100 | 50 | 9 | 100 |

# Lecithin/Sphingomyelin (L/S) Ratio




- Thin-layer chromatography
- First test of fetal lung maturity
- Undeserving gold standard

# L/S Ratio

Advantages

- Good sensitivity for immaturity
- Quantitative
- QC/PT available

Disadvantages

- Large sample volume
- Effected by blood and meconium
- Time consuming
- Technically difficult
- Imprecise (CV ~20%)
- Wide grey zone

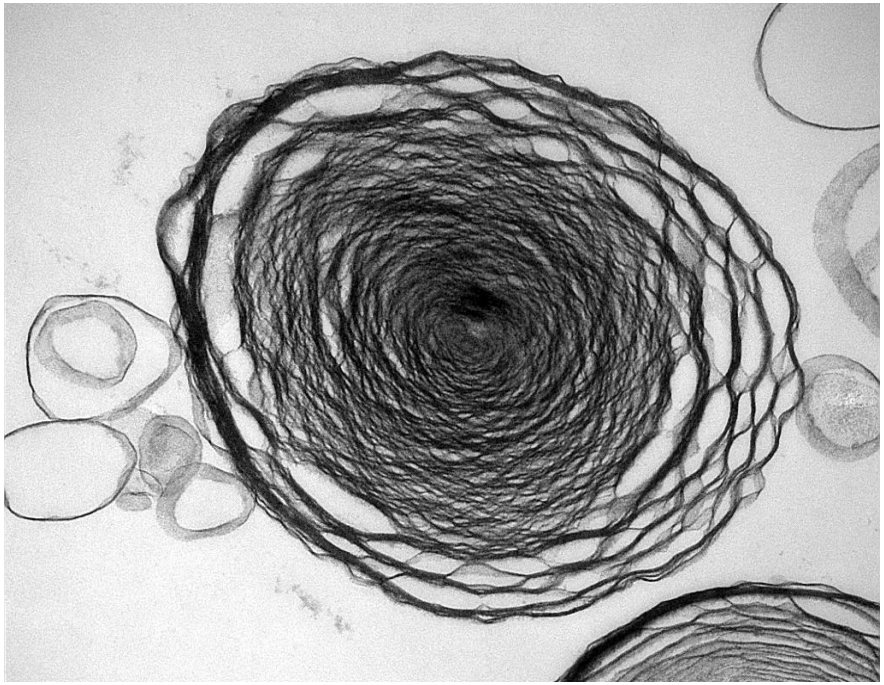
| | |
|---|---|
| Immature | ≤1.5 |
| Transitional | 1.6-2.4 |
| Mature | ≥2.5 |

# L/S Ratio Performance

| Reference | N | Cutoff | Sensitivity (%) | Specificity (%) | Immature PV (%) | Mature PV (%) |
|---|---|---|---|---|---|---|
| *AJOG* 1980 136:222 | 74 | 2.0 | 50 | 93 | 29 | 97 |
| *AJOG* 1982 144:167 | 311 | 2.0 | 90 | 74 | 19 | 99 |
| *Obstet Gynecol* 1984 63:52 | 131 | 2.5 | 100 | 77 | 26 | 100 |
| *Clin Chem* 1984 30:1233 | 47 | 2.0 | 63 | 97 | 83 | 92 |
| *Obstet Gynecol* 1984 63:349 | 205 | 2.0 | 100 | 87 | 31 | 100 |
| *Clin Chem* 1986 32:260 | 88 | 2.0 | 84 | 87 | 57 | 97 |
| *Obstet Gynecol* 1993 82:1004 | 140 | 2.0 | 83 | 60 | 53 | 86 |
| *J Perinat Med* 1996 24:355 | 41 | 2.0 | 100 | 81 | 36 | 100 |

# Lamellar Body Count (LBC)

- LBs similar in size to blood platelets
  - 1 to 5 µm vs. 2 to 4 µm
- Enumerate with automated cell counter



Courtesy of A.M. Gronowski

# Lamellar Body Count

Advantages

- High sensitivity for immaturity
- Quick and simple to perform
- Widely available and inexpensive
- Low sample volume
- PT available

Disadvantages

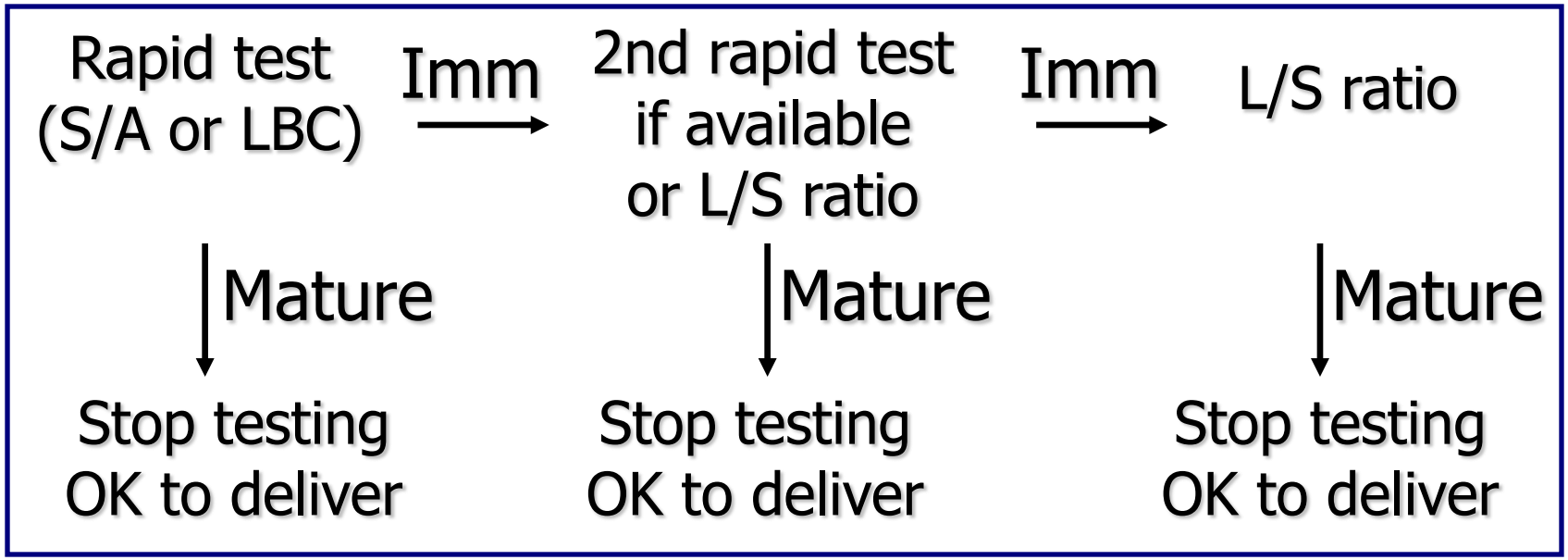
- Effected by blood and meconium
- Lab-developed test
- Instrument-specific cutoffs for maturity
- No commercial QC available

# LBC Performance

| | Reference | RDS/ Total | Cutoff (/μL) | Sensitivity (%) | Specificity (%) | Immature PV (%) | Mature PV (%) |
|---|---|---|---|---|---|---|---|
| Uncentrifuged | *J Reprod Med* 1995 40:260 | 7/62 | <46,000 | 100 | 89 | 54 | 100 |
| | *Int J Gynecol Obstet* 2005 89:19 | 23/52 | <50,000 | 100 | 74 | 64 | 100 |
| | *Arch Gynecol Obstet* 2005 271:325 | 17/102 | ≤37,000 | 95 | 37 | 34 | 98 |
| | *AJCP* 2006 126:894 | 12/184 | <50,000 | 92 | 60 | 14 | 99 |
| Centrifuged | *Obstet Gynecol* 1993 81:619 | 28/247 | ≤55,000 | 100 | 59 | 24 | 100 |
| | *AJOG* 1994 170:72 | 4/24 | ≤30,000 | 100 | 100 | 100 | 100 |
| | *J Perinatol* 1996 16:176 | 14/170 | <50,000 | 100 | 90 | 56 | 100 |
| | *J Obstet Gynecol* 2005 25:257 | 20/80 | <50,000 | 85 | 70 | 48 | 93 |
| | *J Perinat Med* 2005 33:156 | 61/178 | ≤22,000 | 73 | 82 | 66 | 86 |

# Testing Strategies

- A mature result from any FLM test is highly predictive of lung maturity
- Multiple tests should not be ordered routinely




ACOG Educational Bulletin #230 (1996)

# Contemporary Issues

# ACOG PRACTICE BULLETIN

CLINICAL MANAGEMENT GUIDELINES FOR OBSTETRICIAN–GYNECOLOGISTS

NUMBER 97, SEPTEMBER 2008

*Replaces Educational Bulletin Number 230, November 1996*

# Fetal Lung Maturity

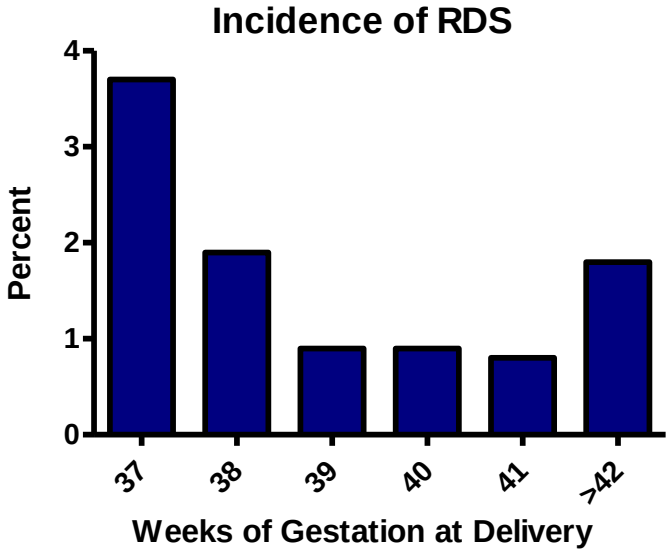
# Sequential Test Strategy

- Acknowledges high mature predictive values of all FLM tests
- No longer specifically mentions sequential testing
- Select FLM test on basis of specimen quality
  - Presence or absence of contaminants

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 8, 2009 VOL. 360 NO. 2

# Timing of Elective Repeat Cesarean Delivery at Term and Neonatal Outcomes

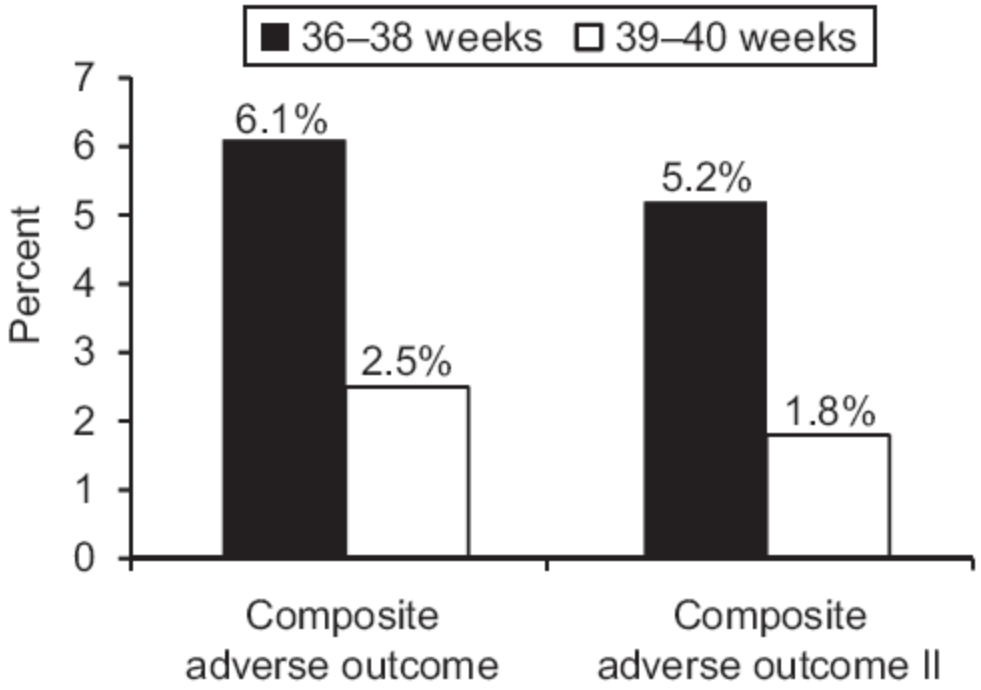



- 13,258 C-sections
- Delivery before 39 weeks associated with adverse respiratory outcomes
- Elective delivery at <39 weeks discouraged unless fetal lungs are mature

# Neonatal Outcomes After Demonstrated Fetal Lung Maturity Before 39 Weeks of Gestation

*Elizabeth Bates, MD, Dwight J. Rouse, MD, MSPH, Merry Lynn Mann, BS, Victoria Chapman, MPH, Waldemar A. Carlo, MD, and Alan T. N. Tita, MD, PhD*

*(Obstet Gynecol 2010;116:1288–95)*




- Two cohorts
  - 36-38 weeks with mature FLM test result (N=459)
  - 39-40 weeks (N=13,339)
- Delivery before 39 weeks associated with increased risk of adverse outcome
- *Are FLM tests necessary?*

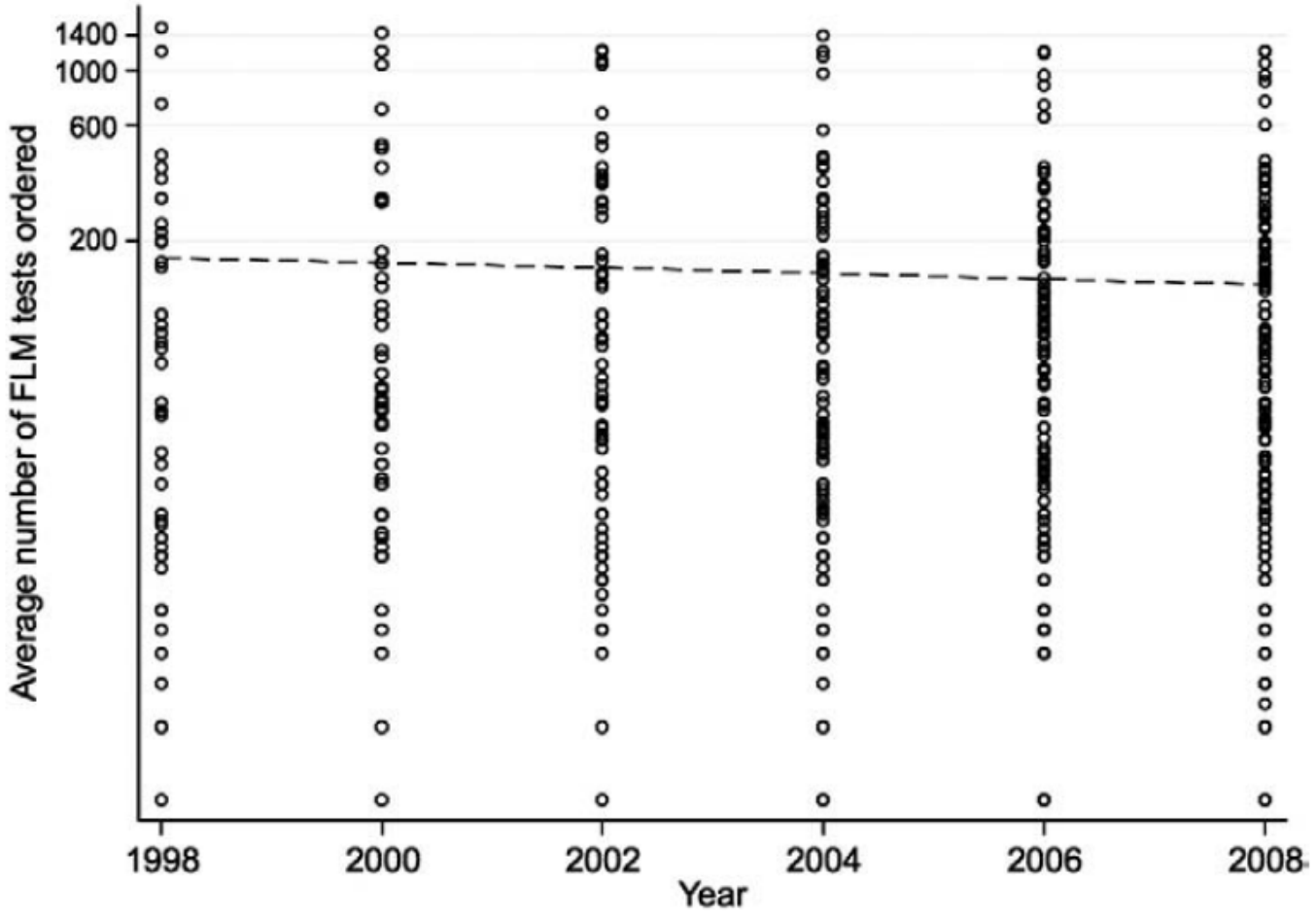
ORIGINAL ARTICLE

# Changing patterns of fetal lung maturity testing

Journal of Perinatology (2007), 1–4

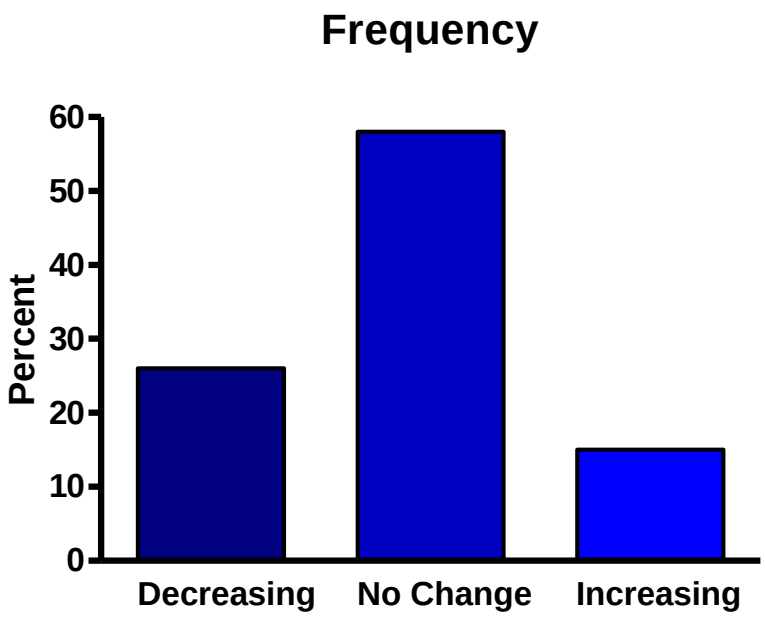
- Noted a decline in FLM tests performed in their lab
- Surveyed SMFM docs regarding FLM test utilization
  - 60% indicated ordering had decreased
  - 14% indicated no change in ordering
  - 24% indicated ordering had increased
- Tests “not needed for patient care” was most common response as to the decreased utilization

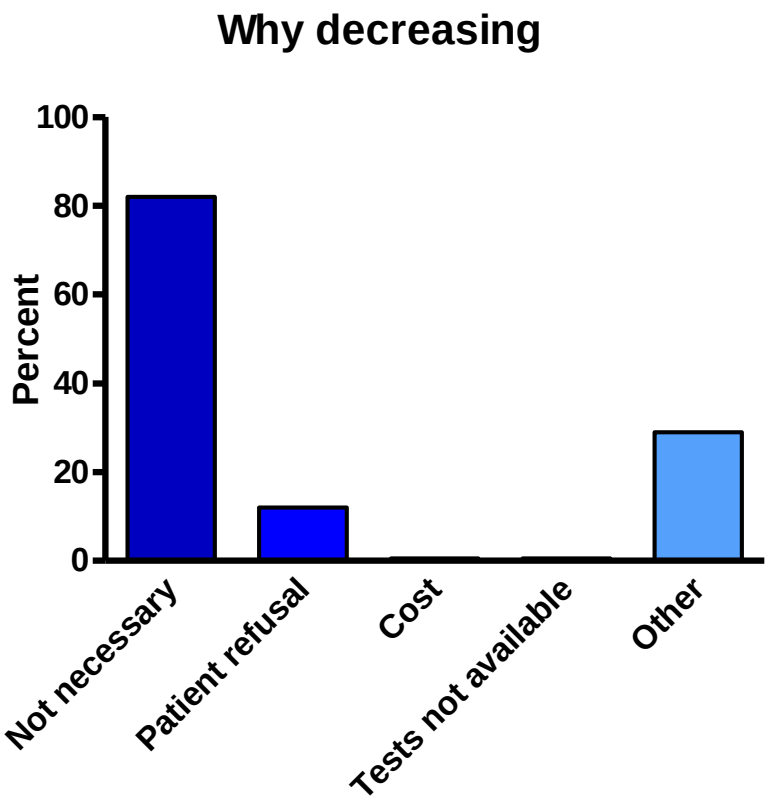
# What is the annual number of FLM tests your lab performed each year? (N=118)




Grenache, et al. *Clin Chim Acta* 2010 411:1746-49

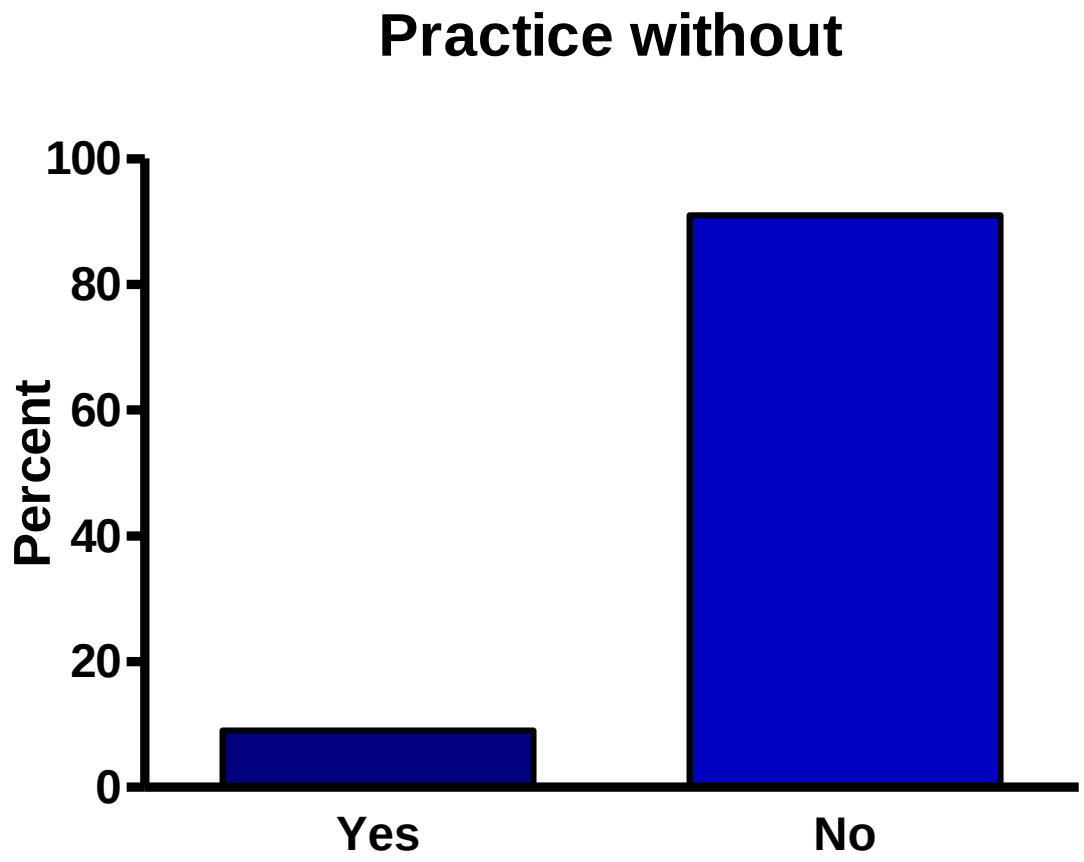
# Is the frequency with which you are ordering FLM tests increasing, decreasing, or staying the same? (N=81)



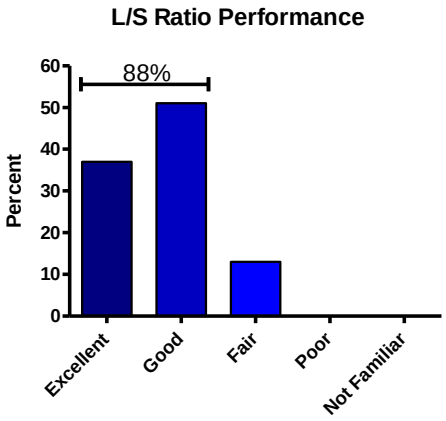

Grenache, et al. *Clin Chim Acta* 2010 411:1746-49

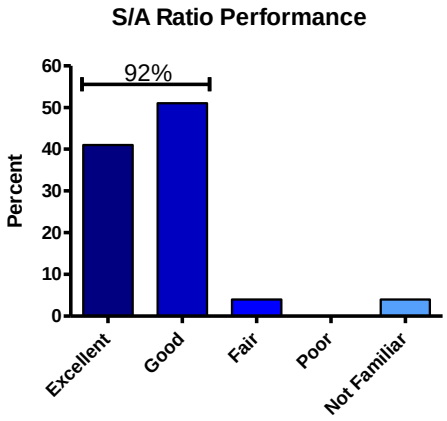
# Could you provide your current level of care without any FLM test results? (N=81)

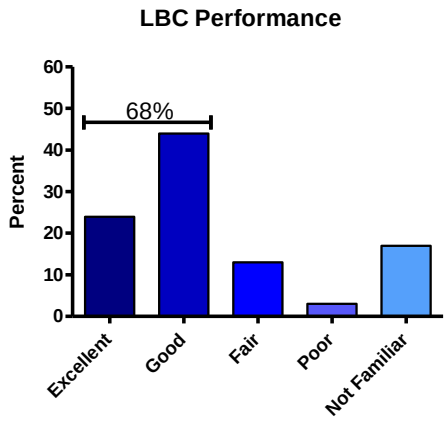


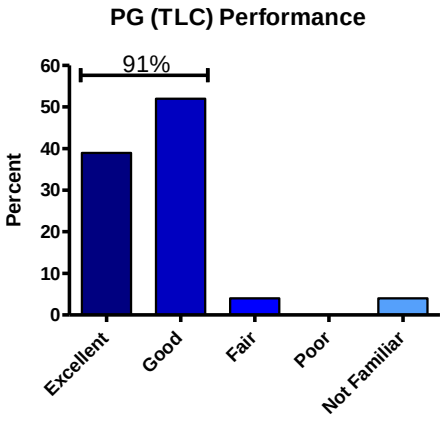

Grenache, et al. *Clin Chim Acta* 2010 411:1746-49

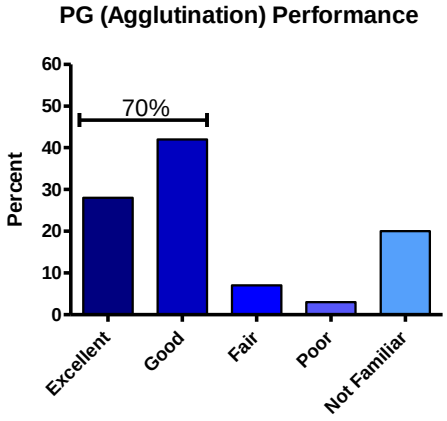
# Indicate the performance of FLM tests based on your impression of their clinical accuracy. (N=81)

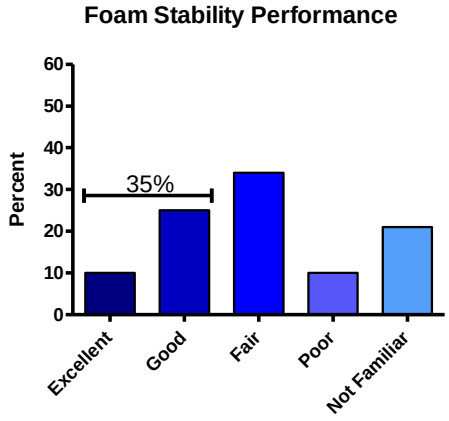


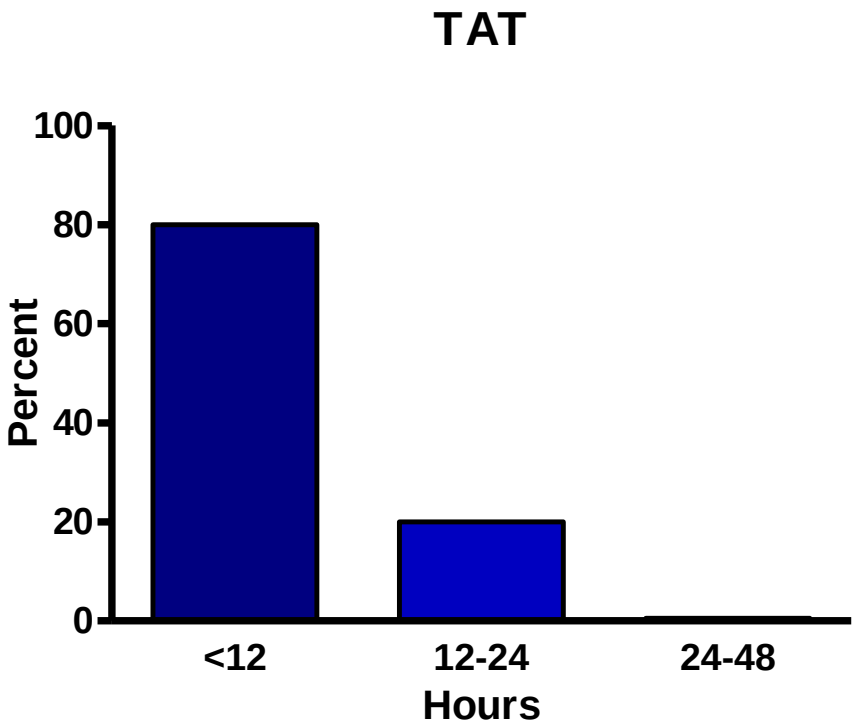

Grenache, et al. *Clin Chim Acta* 2010 411:1746-49

# Loss of S/A ratio

- In 2008 Abbott Diagnostics announced difficulty in maintaining support of their “legacy systems” including the TDx
  - IMx, TDx, TDxFLx
- Retirement of assays performed on these platforms underway
- S/A ratio (marketed as Fetal Lung Maturity II) will be available through the end of 2011
- What might this mean for labs?

# Refer FLM test requests to a performing lab

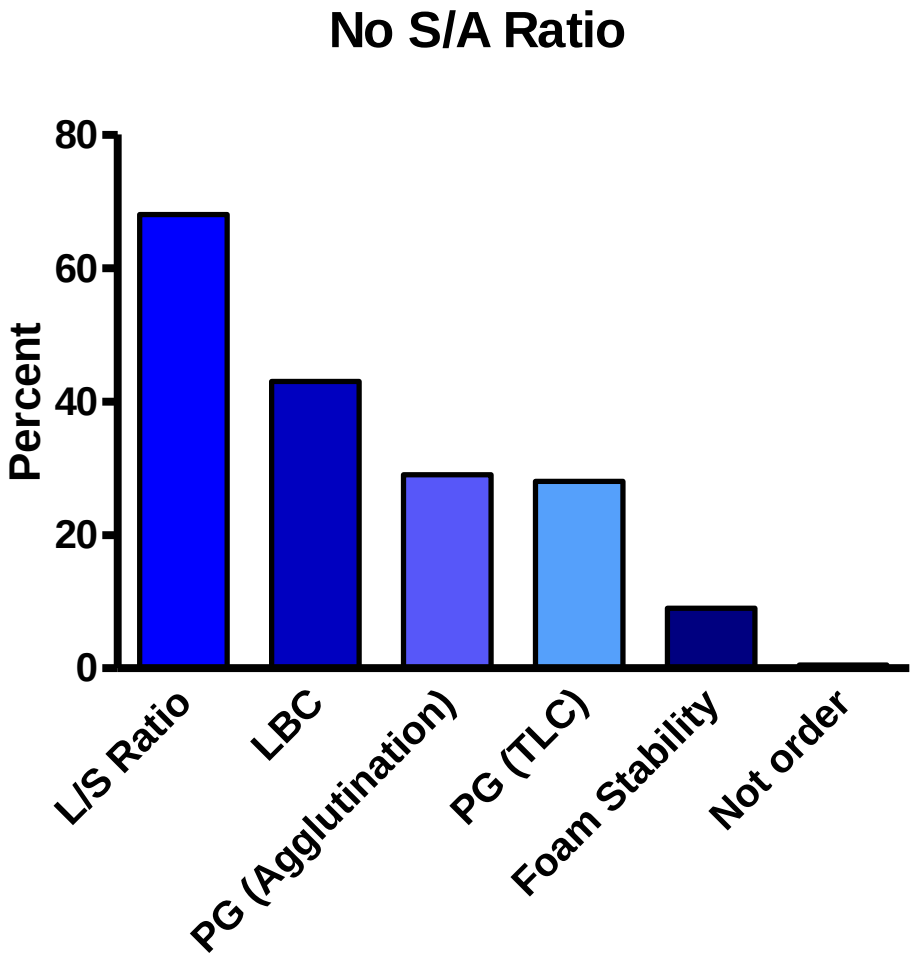
- Within how many hours do you require FLM test results?




Grenache, et al. *Clin Chim Acta* 2010 411:1746-49

# Provide an alternate FLM test

- Which FLM test would you likely order if the S/A ratio were no longer available?



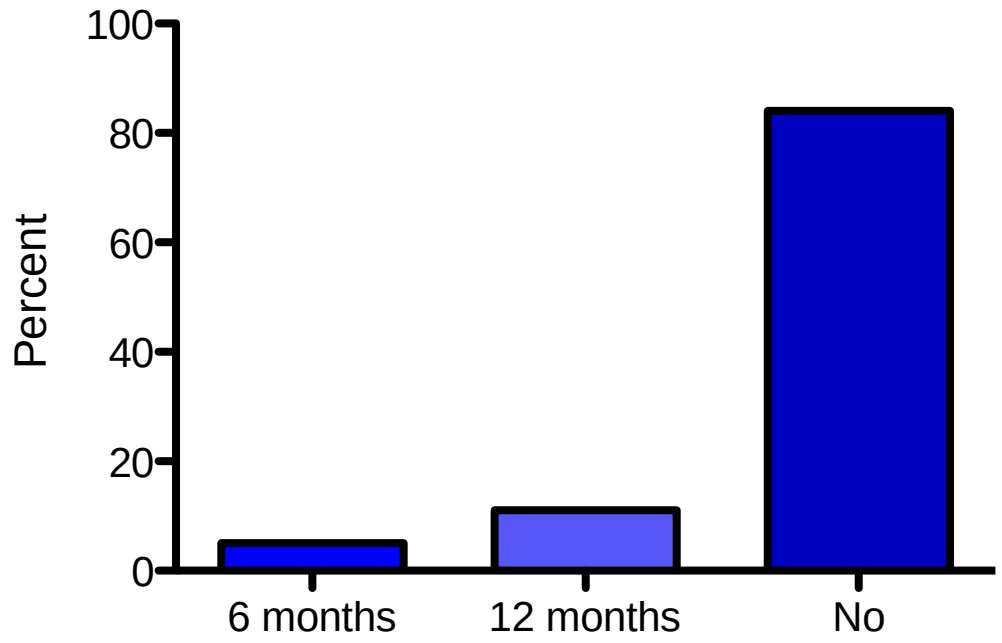

Grenache, et al. *Clin Chim Acta* 2010 411:1746-49

# Alternate FLM Test Considerations

- L/S ratio
  - Not recommended unless ≥15 specimens tested per week (Ashwood ER *Clin Chem* 1997 43:211)
- PG
  - Late marker of maturity
  - History of supply issues with manufacturer of agglutination test
- Foam stability
  - Home brew, qualitative/semi-quantitative result
  - Difficult to maintain reagent integrity
- LBC
  - Home brew, quantitative result
  - Instrumentation widely available

# Do you plan to perform the LBC test?

(N=19)




Top reasons for not performing:

1. Decreasing demand for FLM tests
2. Not familiar with the test
3. Lack of commercial QC material; lack of "approval" from national/provincial government authority

# Summary

- RDS is a serious problem in premature infants but its incidence is decreasing steadily
- All currently available FLM tests are good to excellent predictors of lung maturity but not immaturity
- Infants born before 39 completed weeks, even with biochemical evidence of lung maturity, have poorer outcomes compared to those born after 39 weeks
- In the U.S., the overall frequency of FLM testing appears to be decreasing but tests are not likely to become obsolete
- In Canada, FLM testing is not widely performed
- The loss of the widely used S/A ratio means that laboratories offering FLM tests will need to identify a replacement
- The LBC is the most likely test poised to replace the S/A ratio