ARUP Institute for Learning Presents:

Fetal Lung Maturity Testing: Current Challenges & Considerations

March 30, 2010

David G. Grenache, PhD, MT(ASCP), DABCC, FACB
University of Utah & ARUP Laboratories
Salt Lake City, UT
david.grenache@path.utah.edu

Disclosures

- David G. Grenache has no relevant financial relationships to disclose.

Objectives

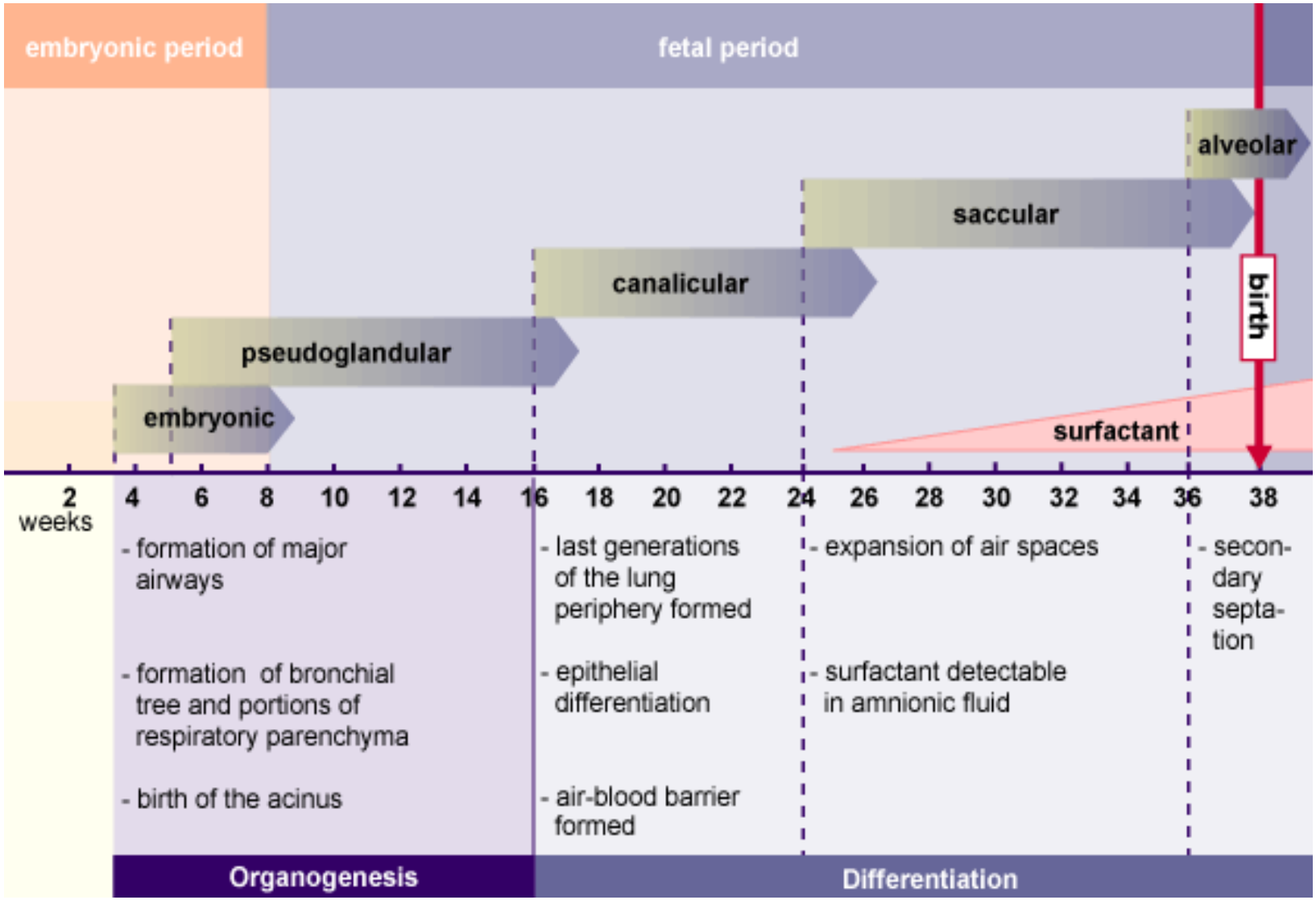
1. Describe the pathophysiology and treatment of respiratory distress syndrome

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 tests

4. Plan a successful validation of the lamellar body count test

Fetal Lung Development

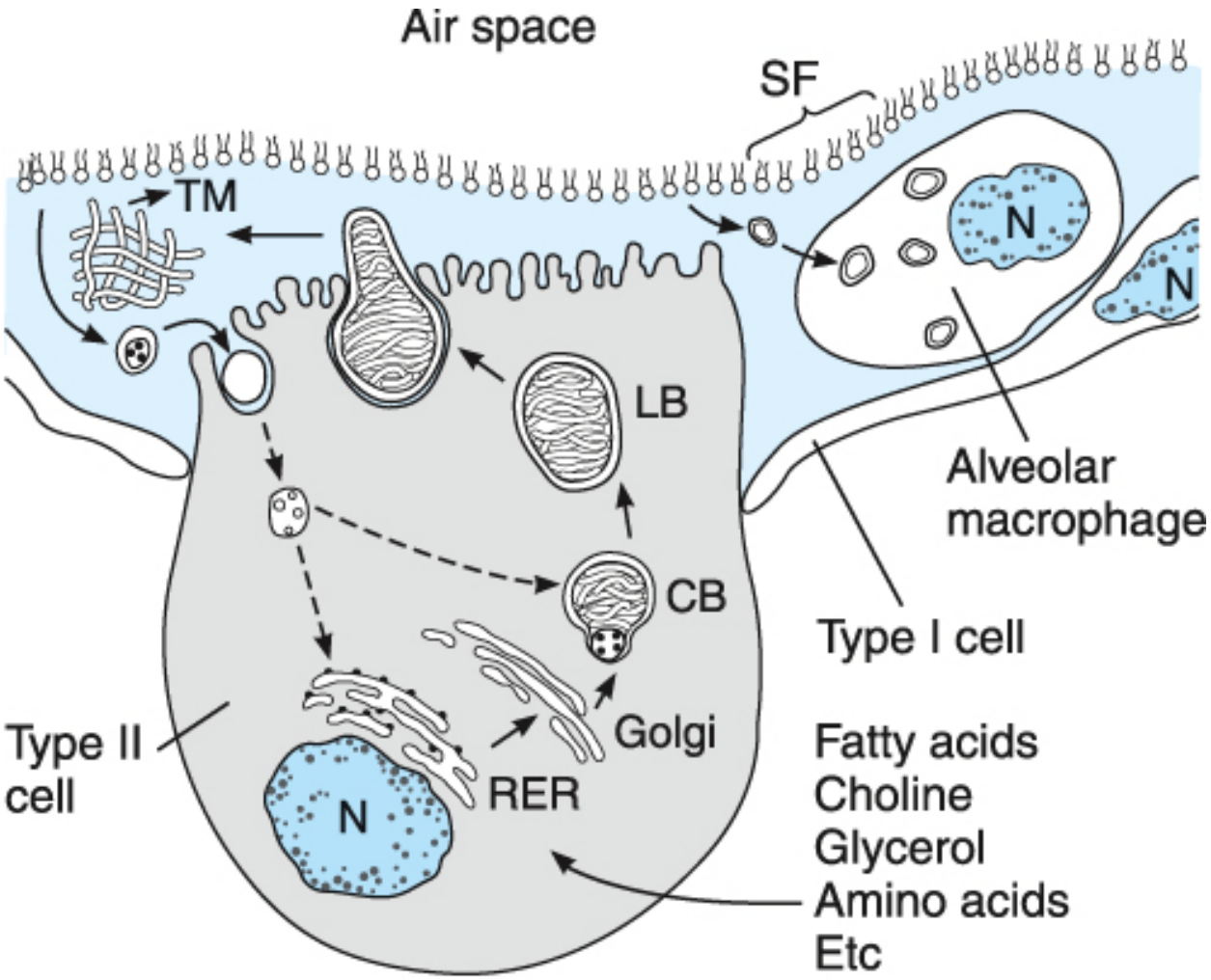


http://www.embryology.ch/anglais/rrespiratory/phasen01.html

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

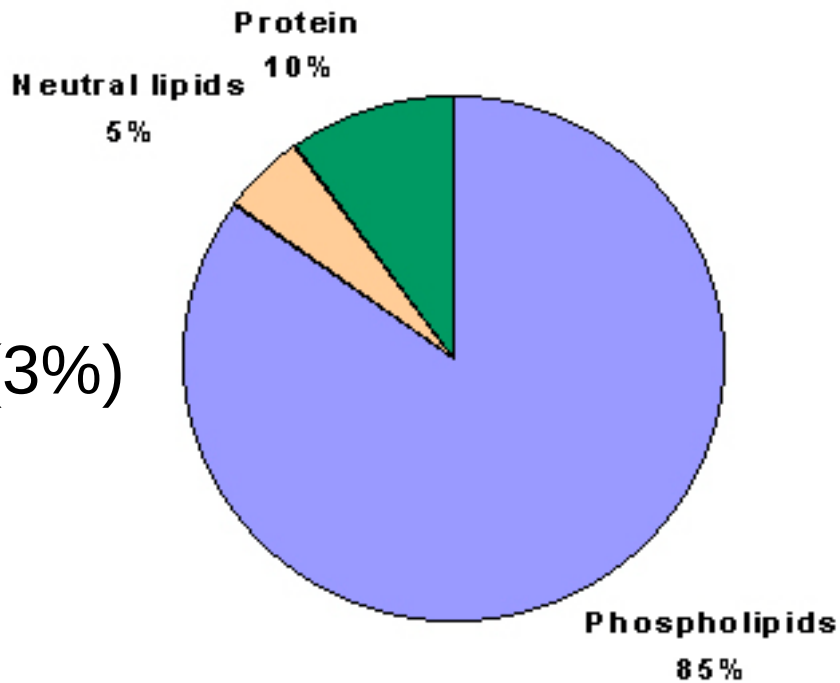
Surfactant Metabolism



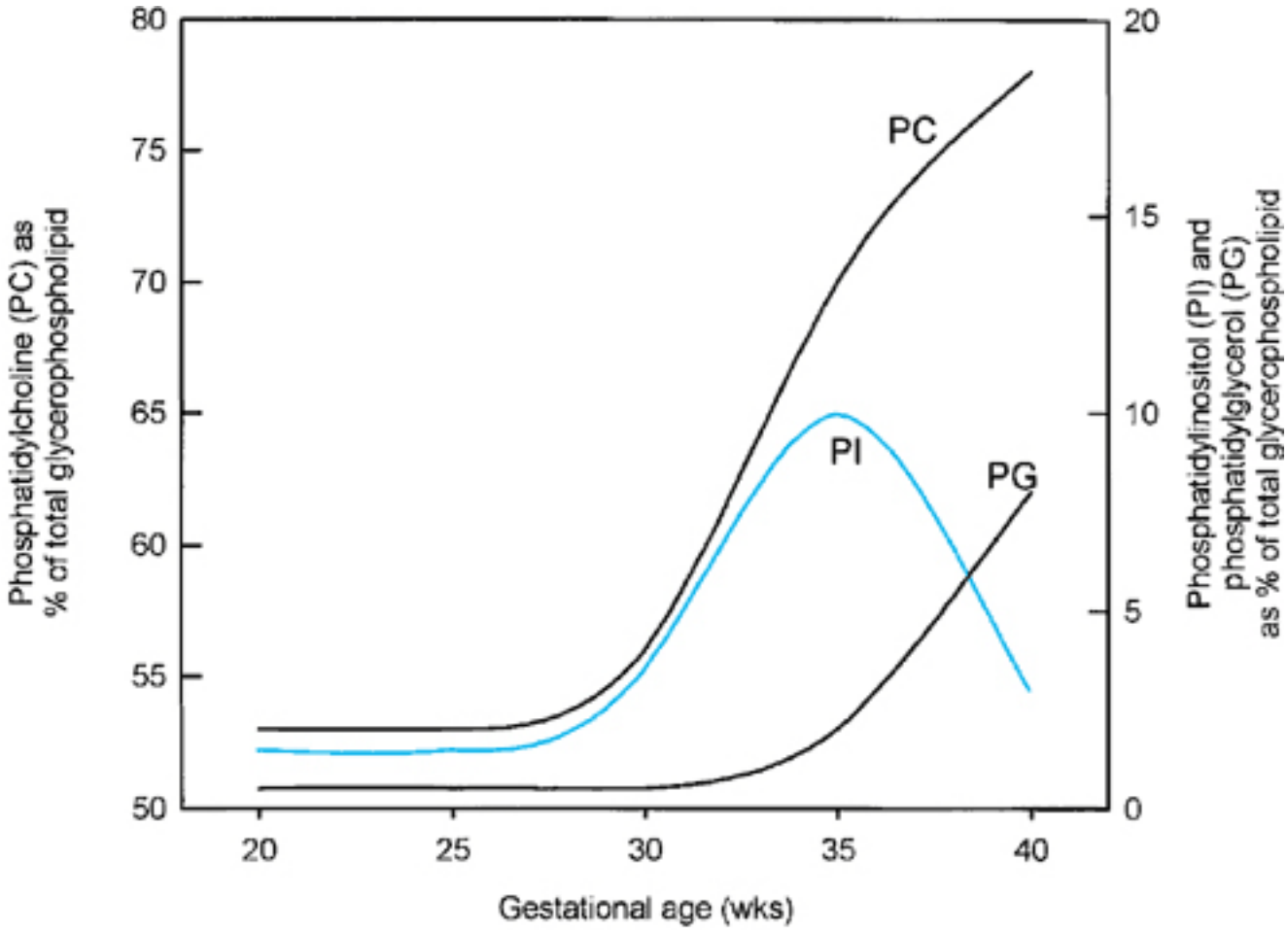
Am Rev Respir Dis 1987;136:426

Pulmonary Surfactants

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



Temporal Expression of Surfactant



Williams Obstetrics, 22nd edition

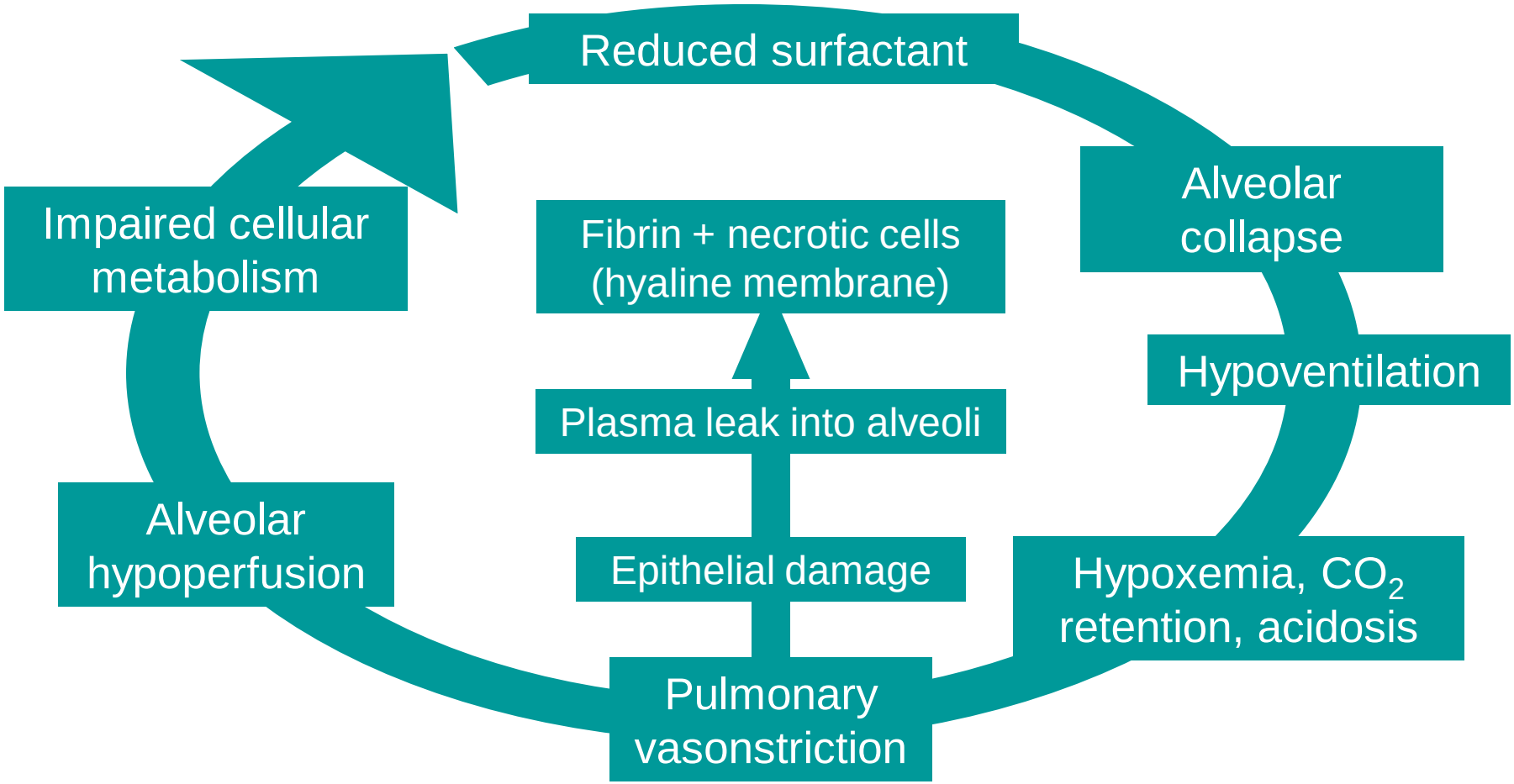
Respiratory Distress Syndrome (RDS)

- Also 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

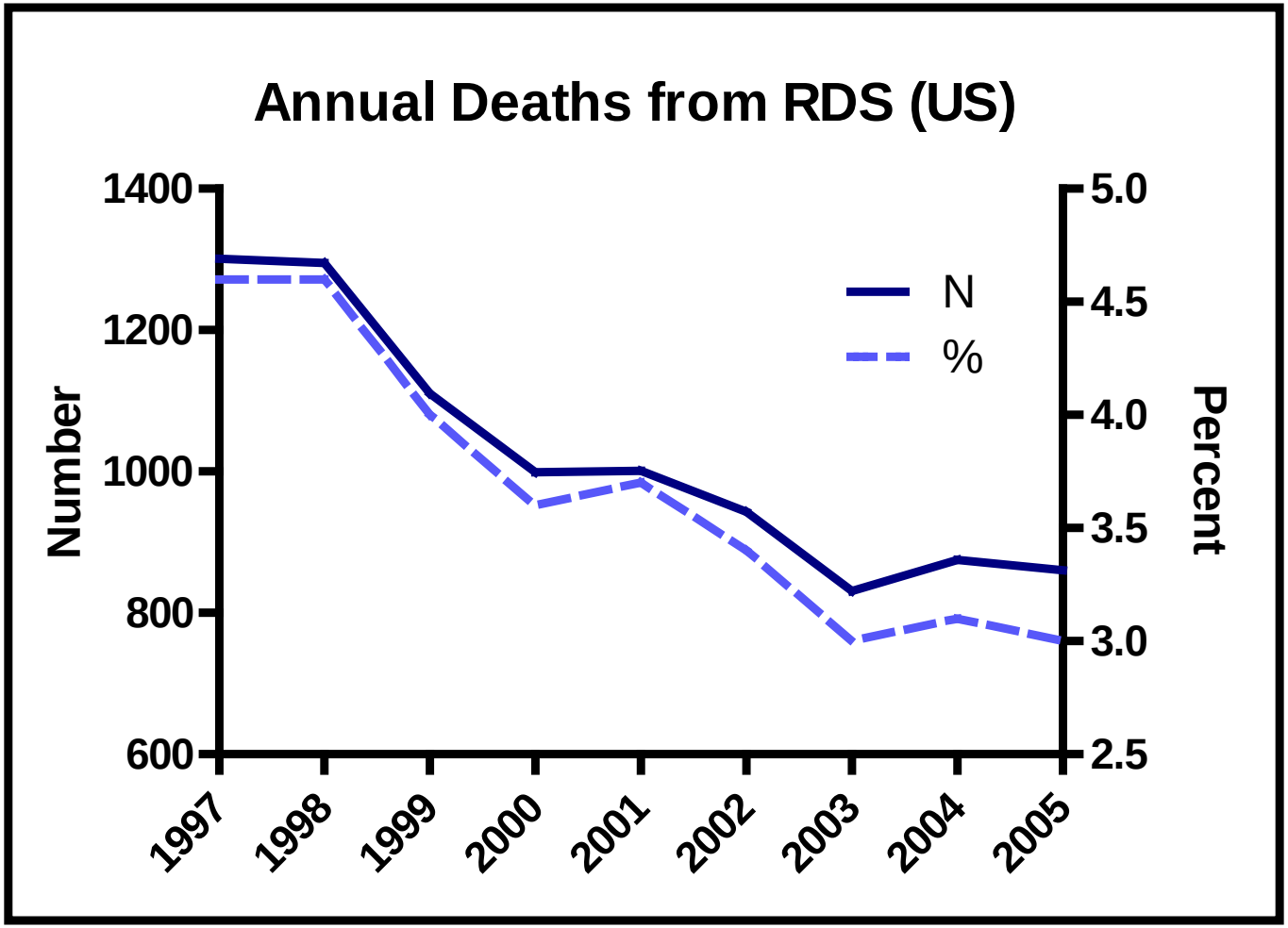
Clinical Manifestations of RDS

- 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 ventilation
  - Keeps alveoli open at expiration

RDS Statistics

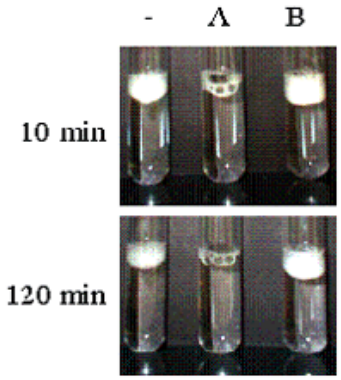


Source: CDC National Vital Statistics Reports

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

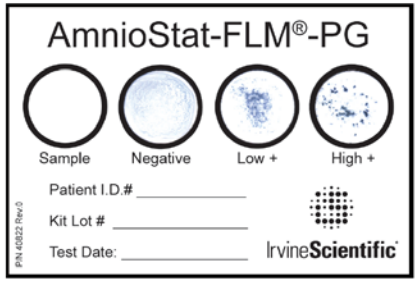
OD 650

S/A Ratio

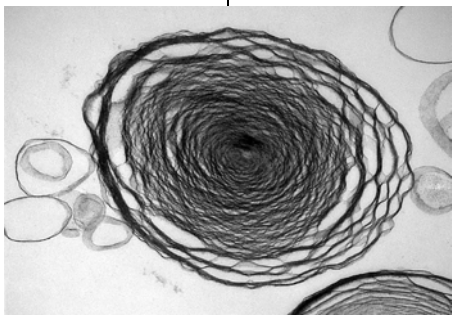
1971 1972 1979 1980 1983 1986 1988

PG by TLC

L/S Ratio

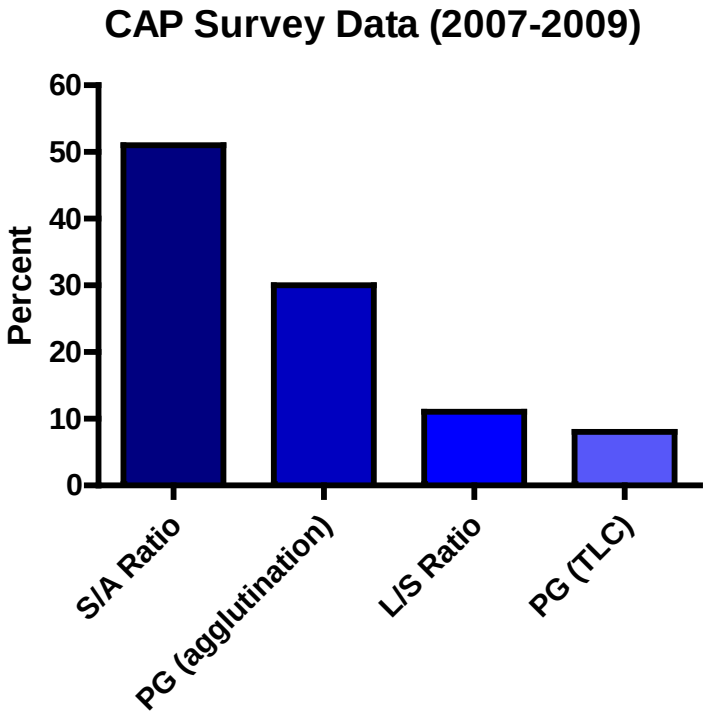


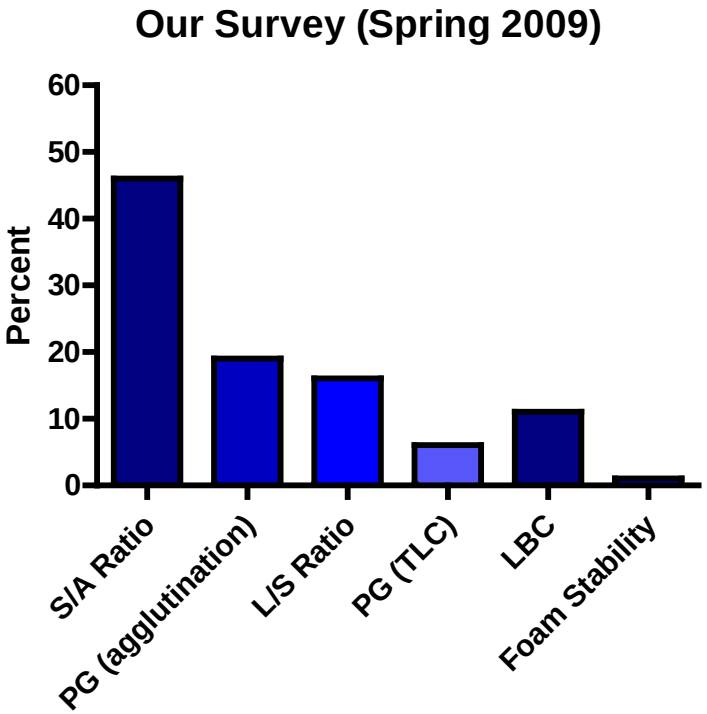
PG by agglutination



Lamellar Body Count

FLM Tests Offered by Labs
Percent of total



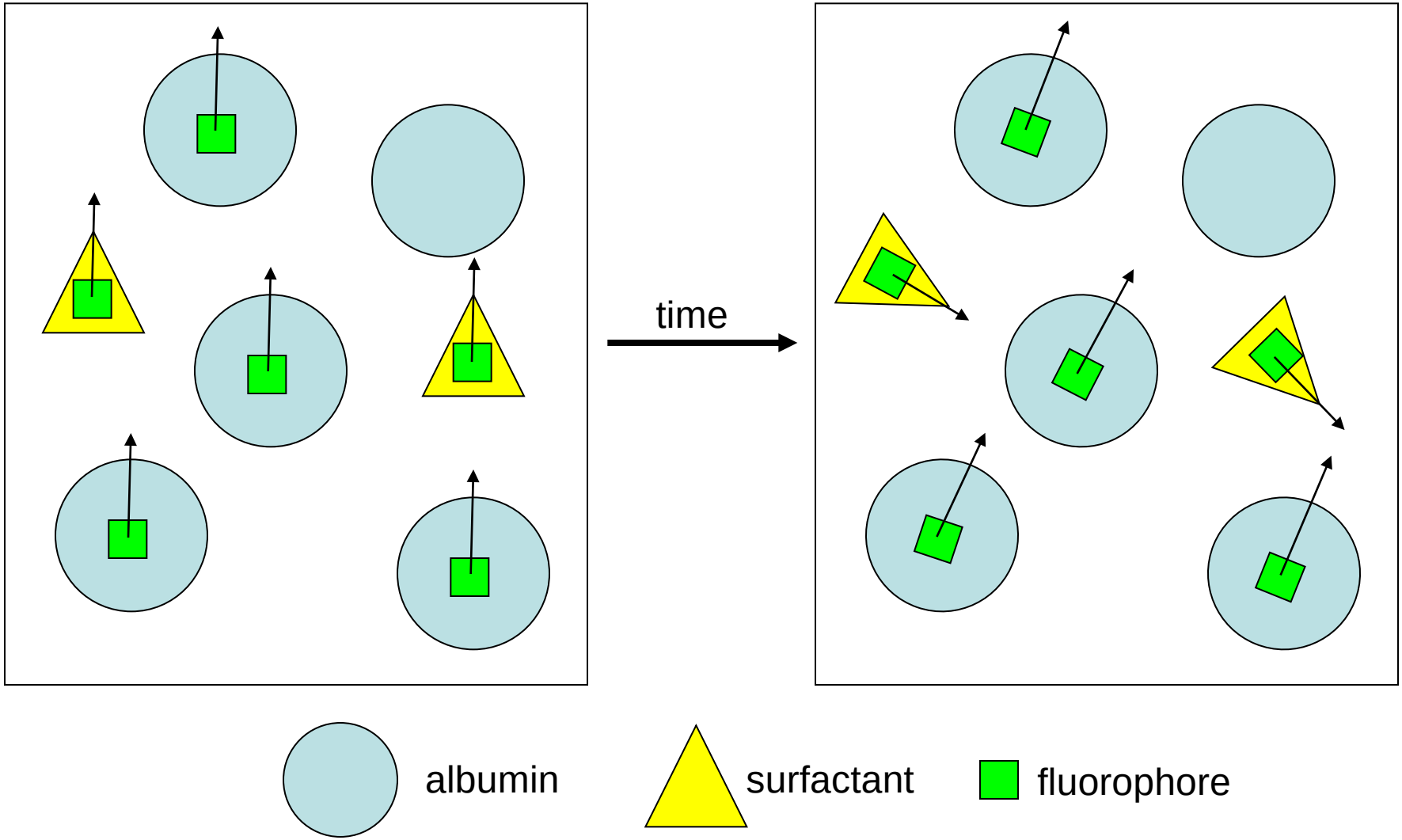


Surfactant/Albumin (S/A) Ratio

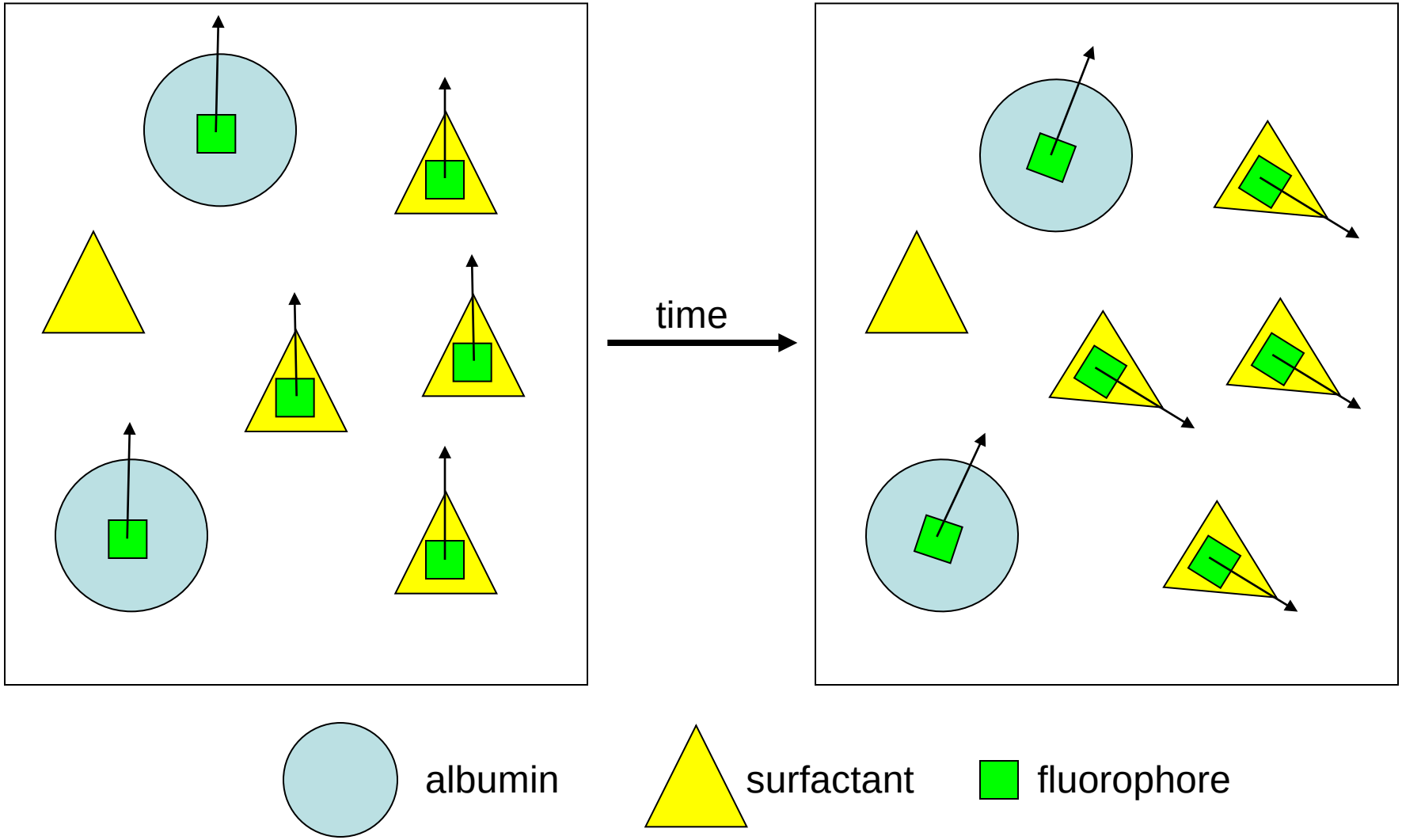
- Fluorescence polarization
  - Decrease in light polarization due to partitioning of a fluorophore between surfactant and albumin

- 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

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

Mature	≥55 mg/g
Indeterminate	40-54 mg/g
Immature	≤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

S/A Ratio and Gestational Age

- Risk of RDS decreases with increasing GA
  - Suggests a single cutoff for maturity is inappropriate

- GA-specific cutoffs improve the interpretation of S/A ratio result

Predicting respiratory distress syndrome using gestational age and fetal lung maturity by fluorescent polarization

Curtis A. Parvin,[a] Lawrence A. Kaplan,[b] John F. Chapman,[c] Timothy G. McManamon,[d] Ann M. Gronowski[a,*]

American Journal of Obstetrics and Gynecology (2005) **192**, 199–207

Table II ORs for RDS relative to a TDx FLM II ratio of 70 mg/g at 37 weeks' GA

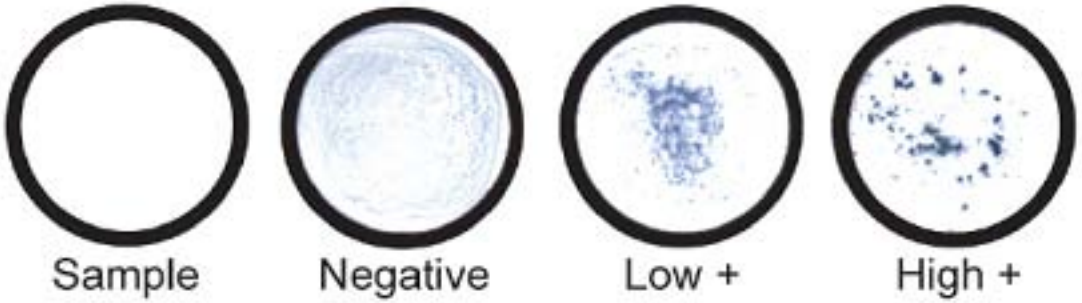
	GA (wk)										
FLM	29	30	31	32	33	34	35	36	37	38	39
10					>1000	>1000	>1000	>1000	837		
20		>1000	>1000	>1000	>1000	818	567	393	273	189	
30	>1000	>1000	799	554	384	266	185	128	88.8	61.6	42.7
40	541	375	260	180	125	86.8	60.2	41.7	28.9	20.1	13.9
50	176	122	84.8	58.8	40.8	28.3	19.6	13.6	9.42	6.54	4.53
60	57.4	39.8	27.6	19.1	13.3	9.21	6.38	4.43	3.07	2.13	1.48
70	18.7	13.0	9.00	6.24	4.33	3.00	2.08	1.44	1.00	0.693	0.481
80	6.09	4.23	2.93	2.03	1.41	0.977	0.677	0.470	0.326	0.226	0.157
90		1.38	0.954	0.662	0.459	0.318	0.221	0.153	0.106	0.074	0.051
100			0.311	0.216	0.149	0.104	0.072	0.050	0.035	0.024	0.017

37 weeks considered term
70=median S/A ratio at 37 wks

Odds Ratio >1 at increased risk
Odds Ratio <1 decreased risk

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

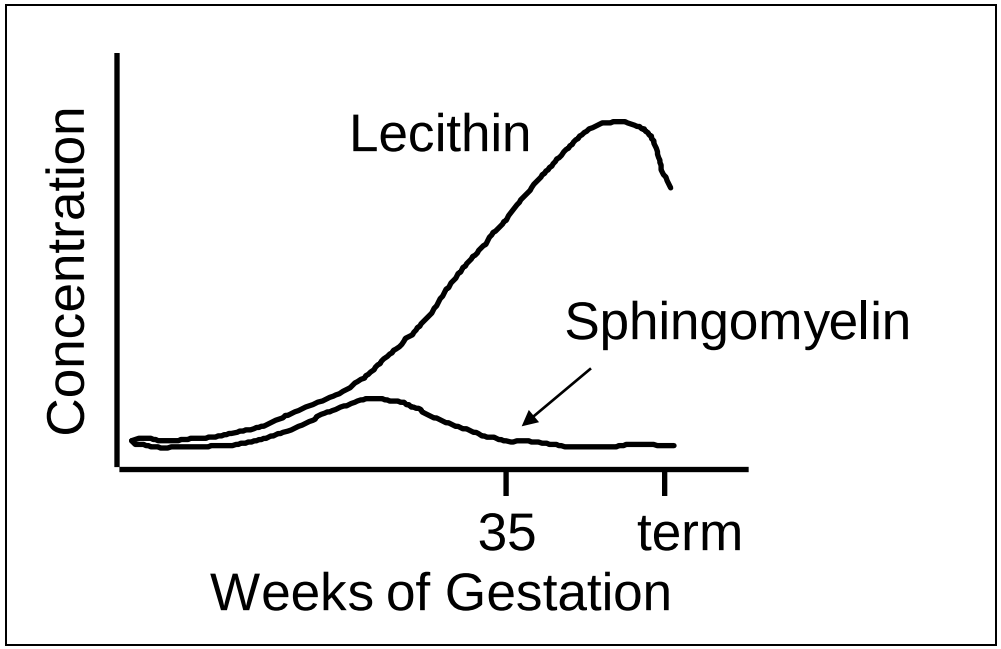
Disadvantages

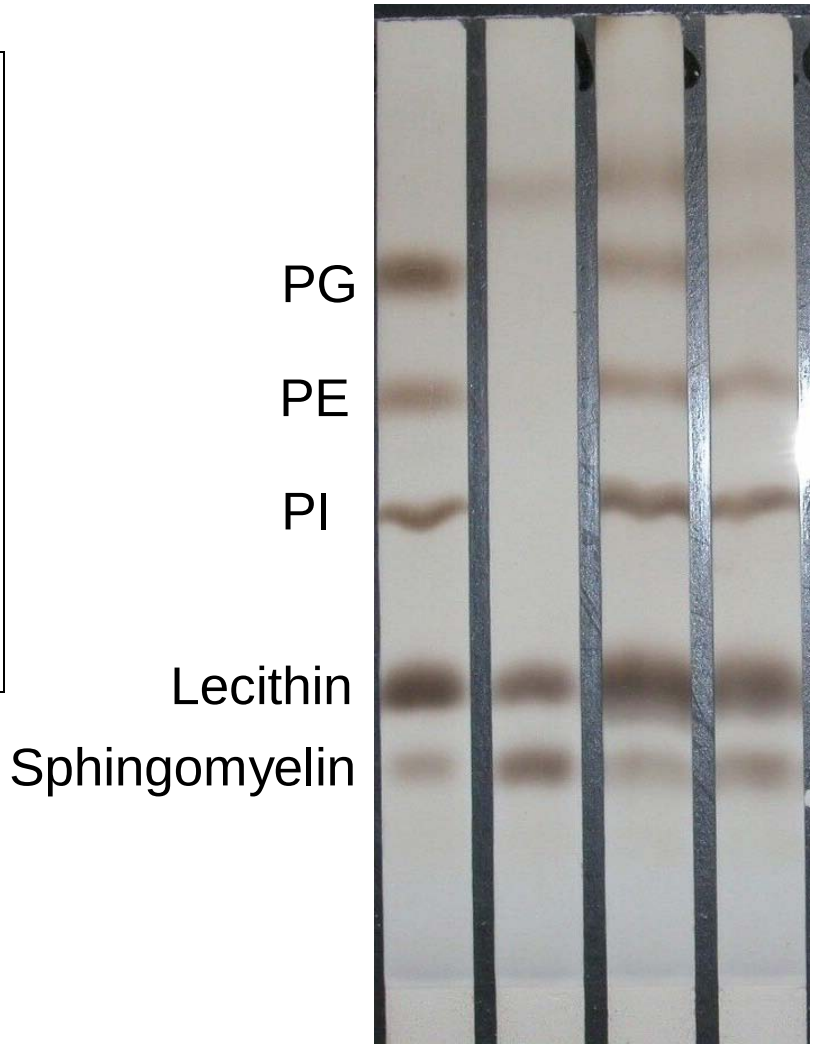
- PG is last surfactant to increase
- Subjective test interpretation
- Single vendor

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

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

Immature	≤1.5
Transitional	1.6-2.4
Mature	≥2.5

Disadvantages

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

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

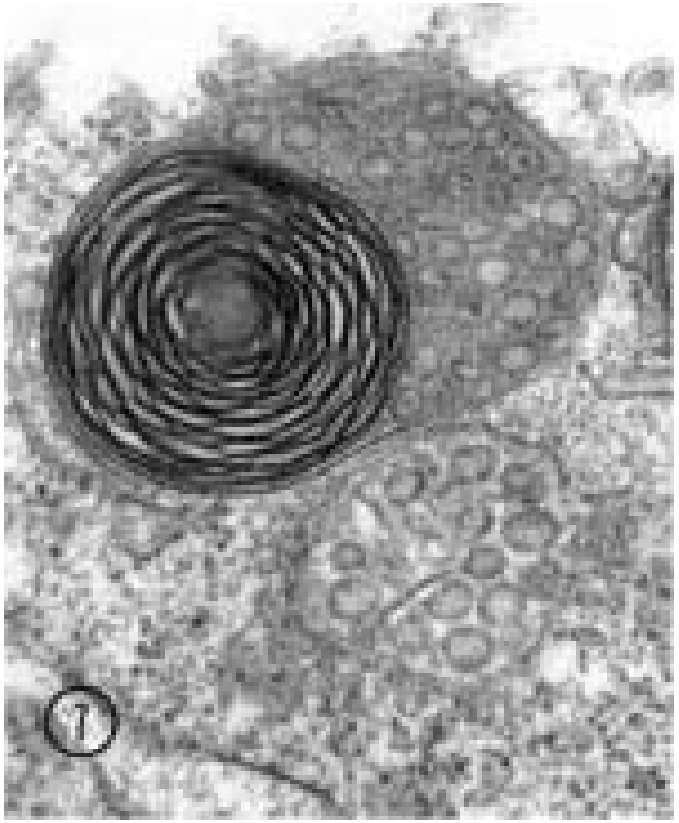
PG by TLC

- Performed as part of the L/S ratio
  - Original method was 2-D TLC
  - 80% of labs performing use 1-D

- Developed to decrease false-immature results of L/S ratio
  - Mature result is L/S ratio greater than cutoff and PG >2% of total phospholipid

Lamellar Body Count (LBC)

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



Lab Invest 2000 80:395–403

LBC Performance

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

Lamellar Body Count

Advantages

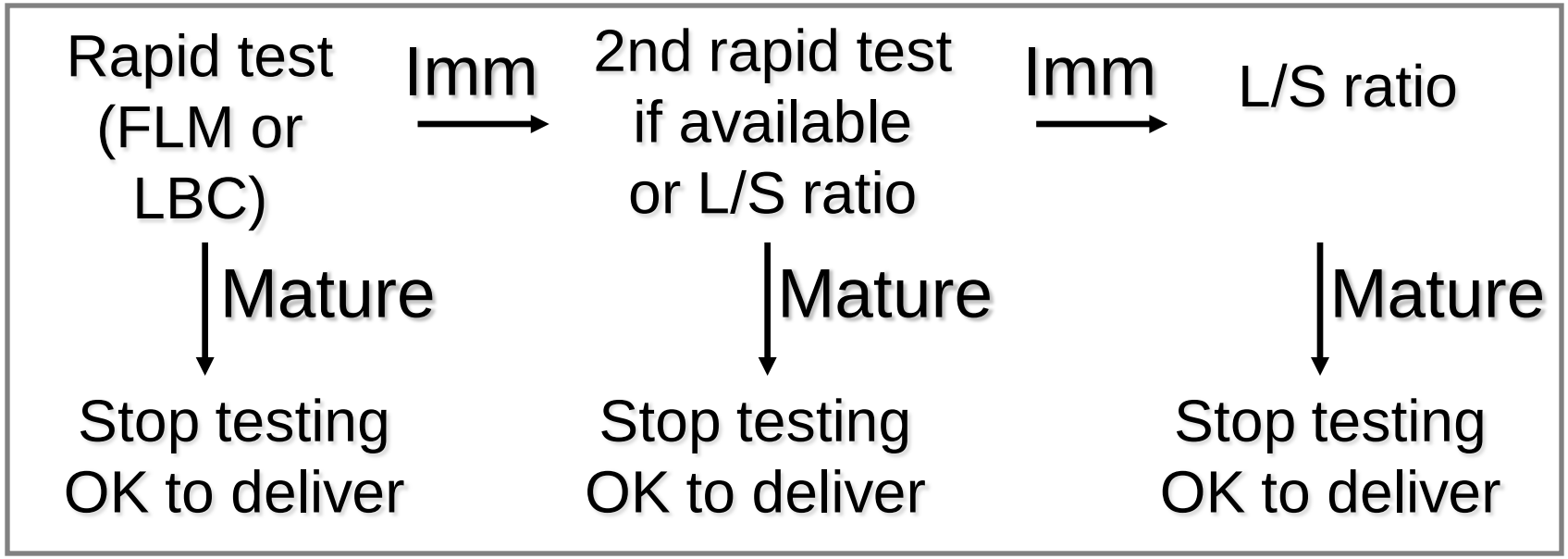
- High sensitivity for immaturity
- Quick and simple to perform
- Widely available and inexpensive
- Low sample volume
- PT available

Disadvantages

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

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

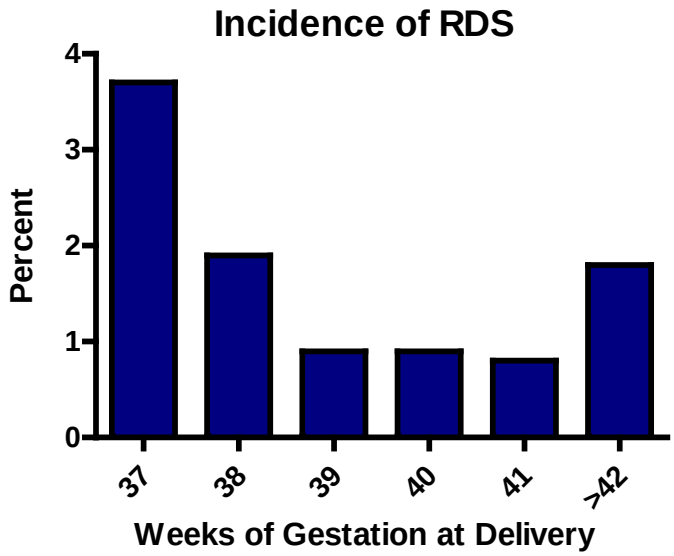
Sequential Test Strategy

- 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

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

Lab Survey

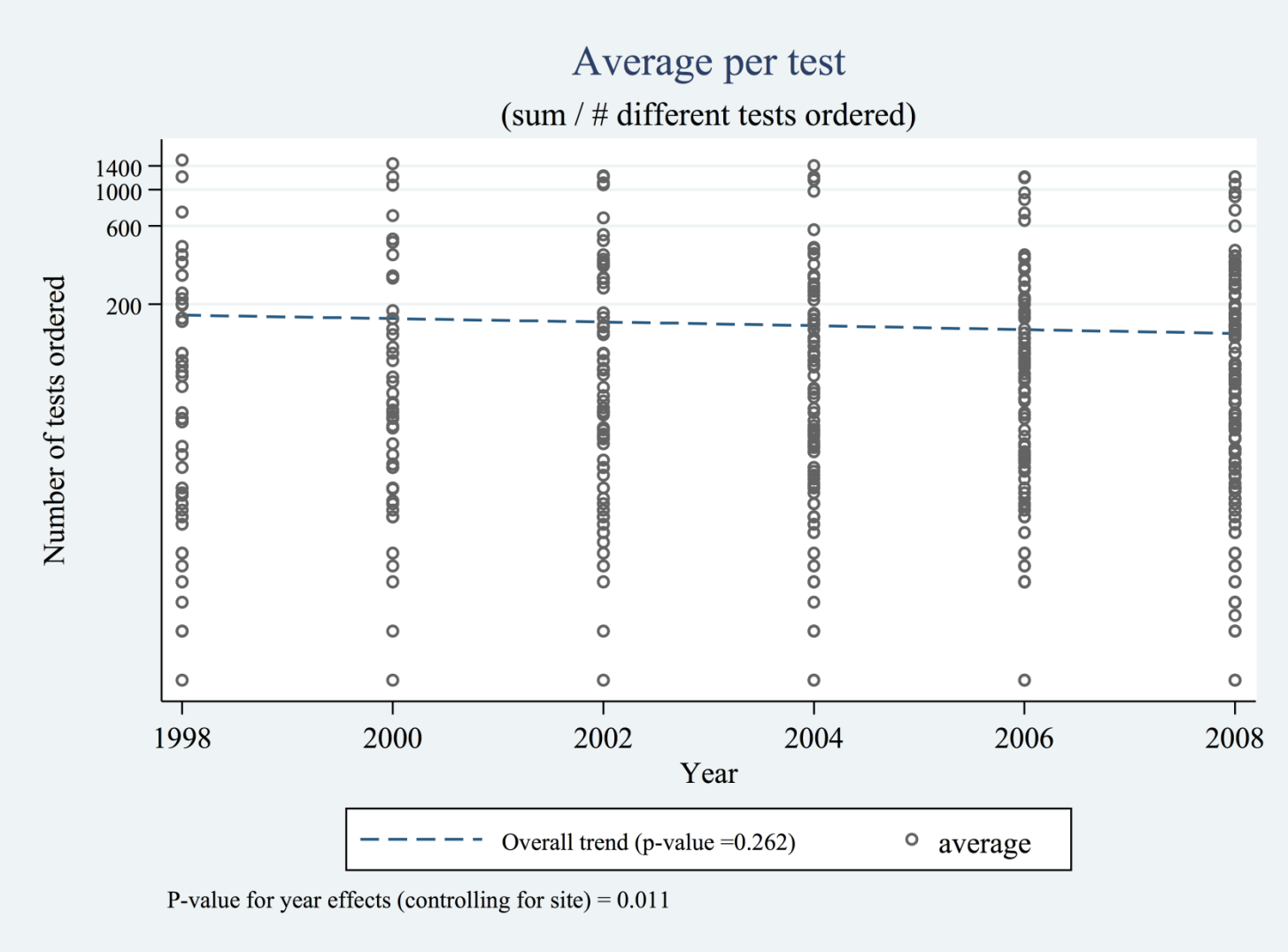
- Spring 2009

- 6,137 AACC Members/CLN Subscribers (USA)
  - Lab Directors, Supervisors, Managers
  - 251 responses (4.1%)

- What is the annual number of FLM tests your lab performed each year by test?
  - 118 responses (2.0%)

Unpublished data

Decreasing Utilization of FLM Tests



Unpublished data

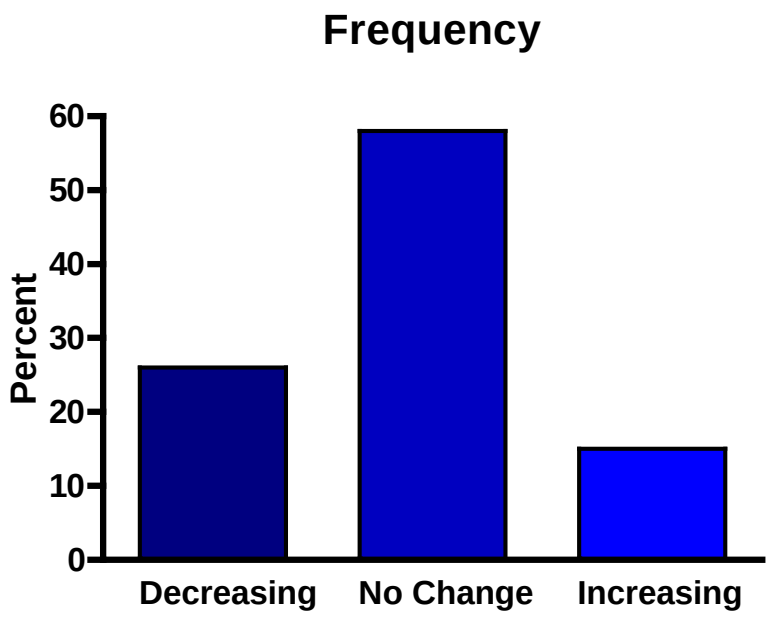
OB/Gyn Survey

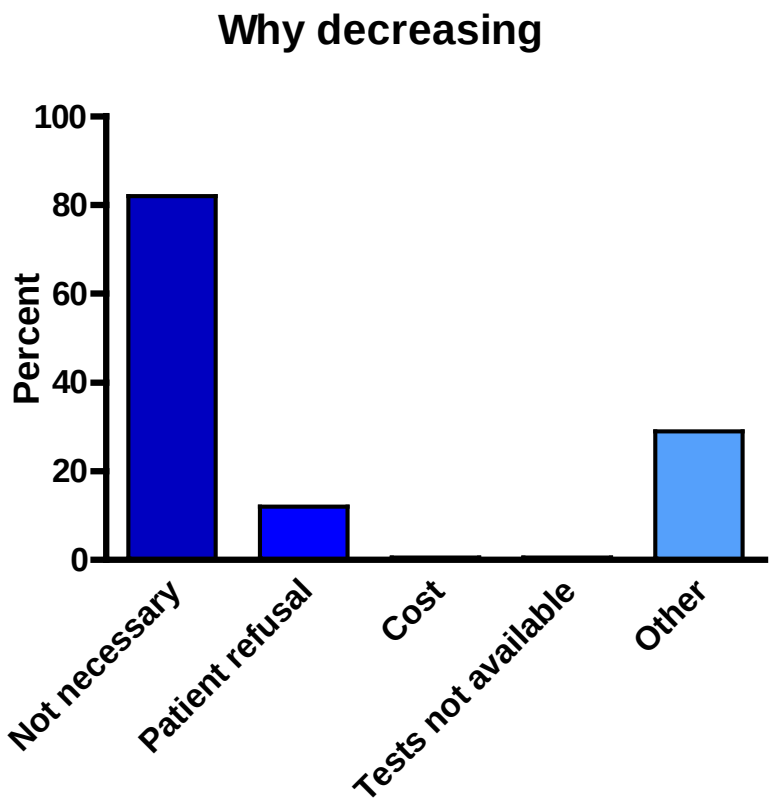
- Spring 2009

- 2,067 Fellows of the Society for Maternal-Fetal Medicine (USA)
  - 66 responses (3.2%)

Unpublished data

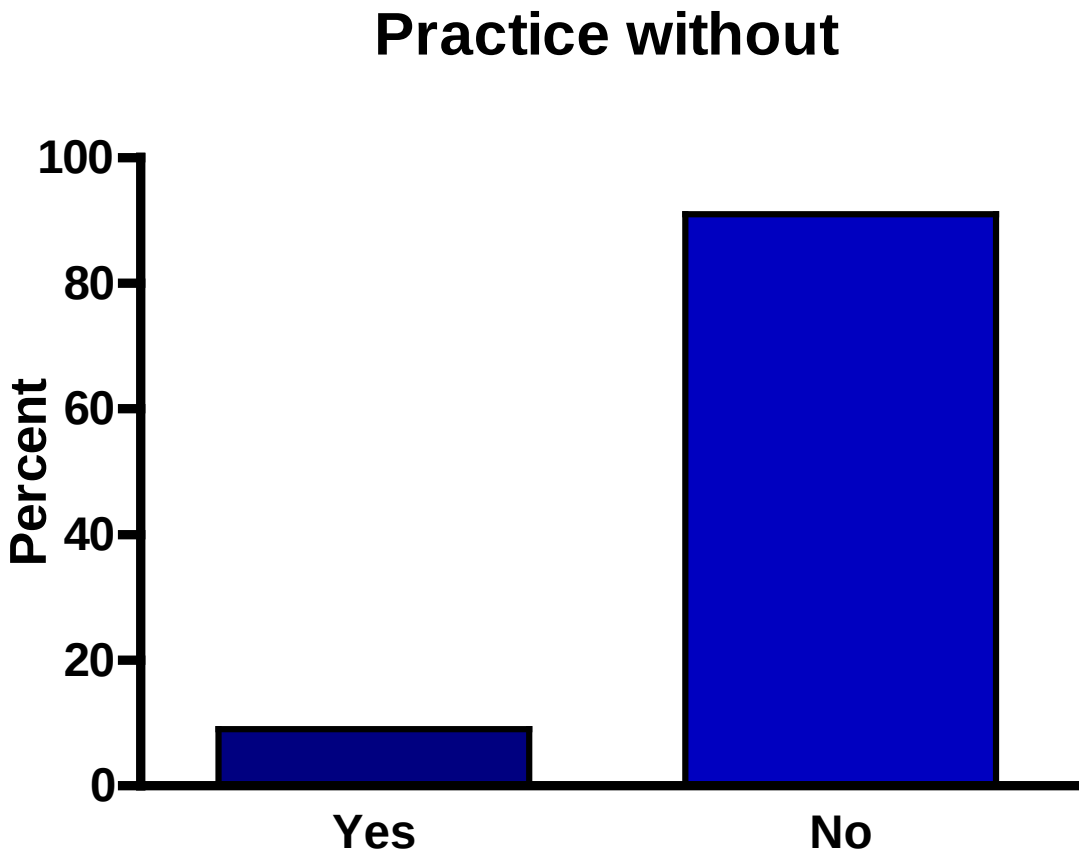
Is the frequency with which you are ordering FLM tests increasing, decreasing, or staying the same?





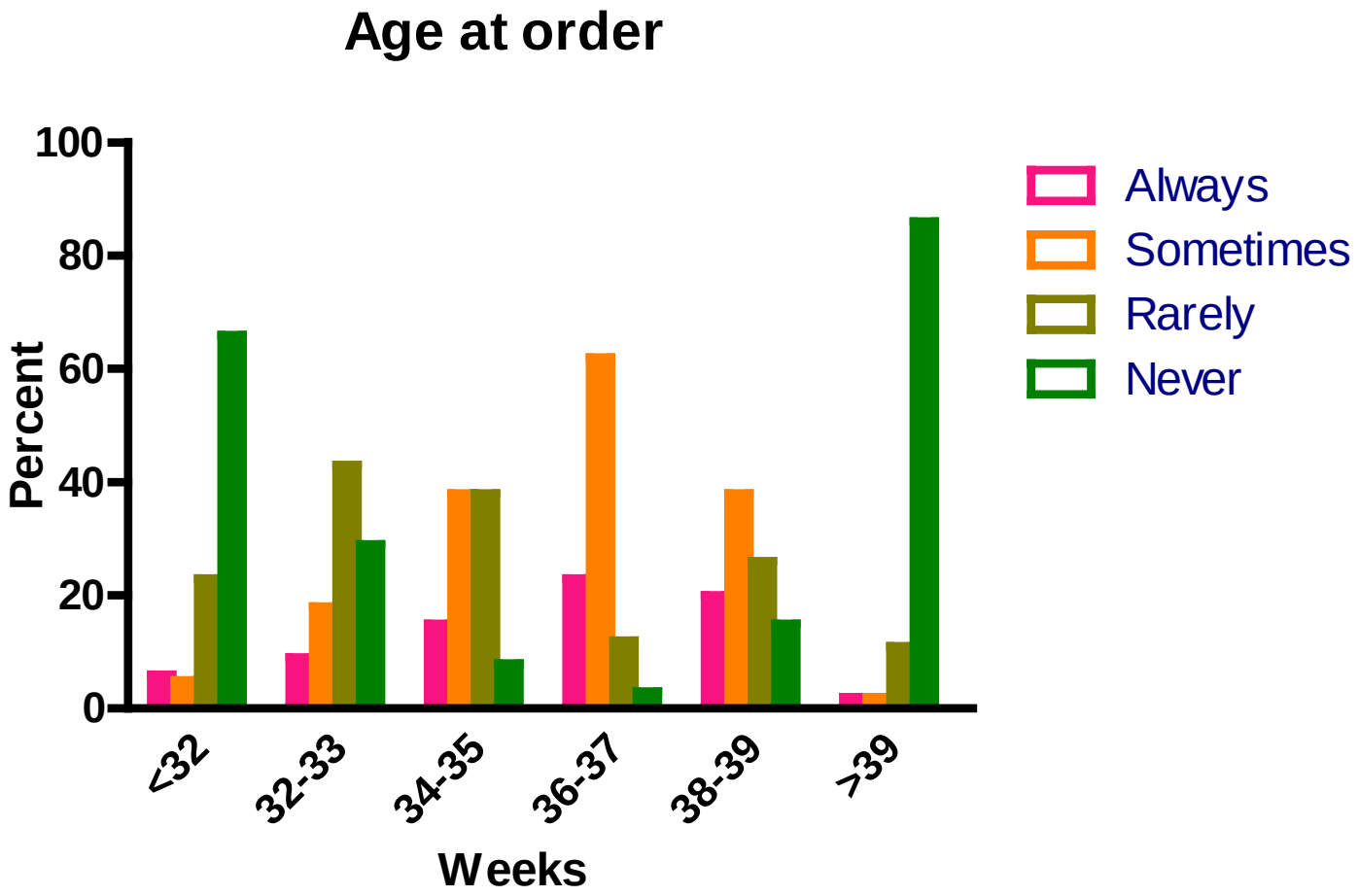
Unpublished data

Could you provide your current level of care without any FLM test results?



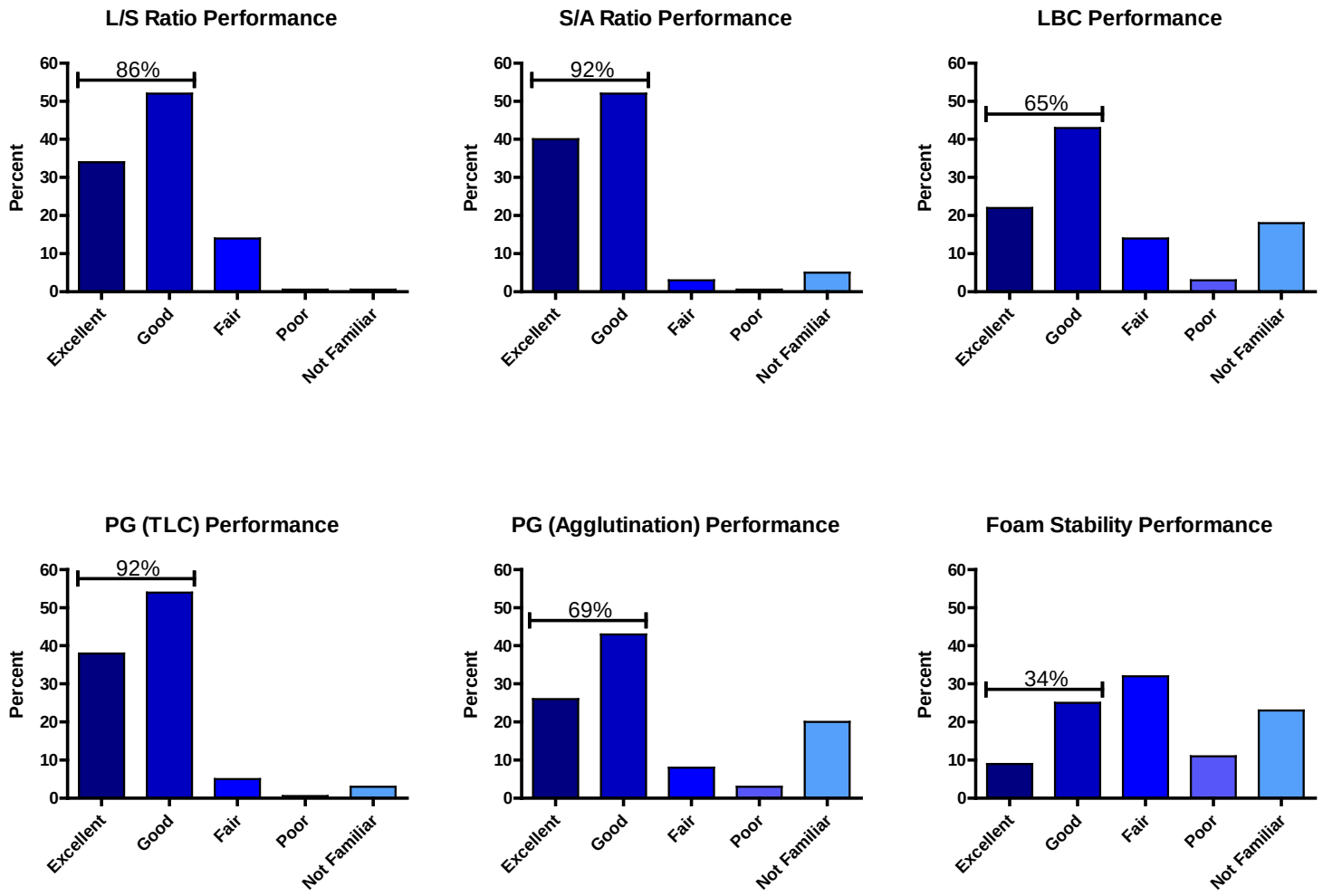
Unpublished data

Based on gestational age, how frequently do you order FLM tests?



Unpublished data

Indicate the performance of FLM tests based on your impression of their clinical accuracy.



Unpublished data

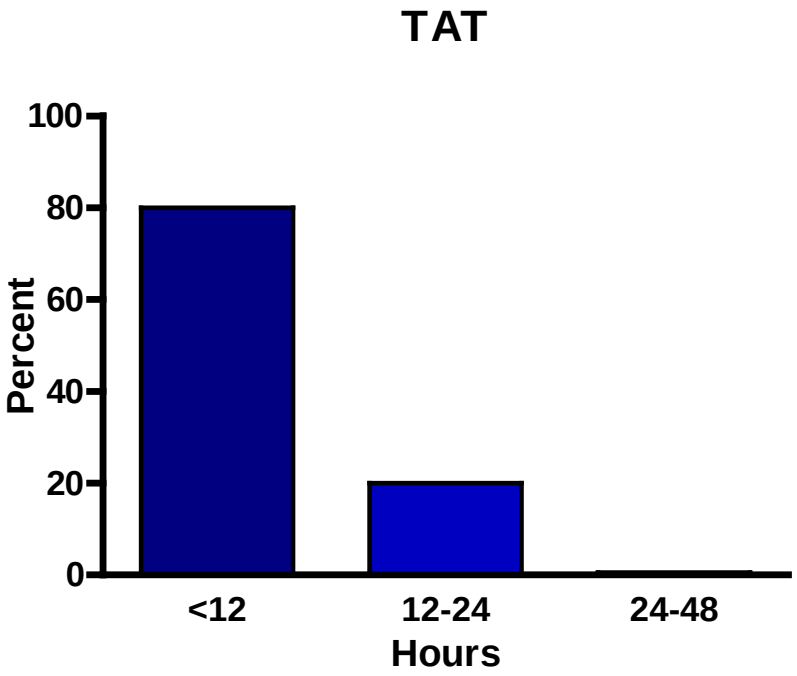
Loss of S/A ratio?

- In 2008 Abbott Diagnostics announced difficulty in maintaining support of their “legacy systems” including the TDx
- Retirement of assays performed on these platforms underway
- No formal announcement regarding the fate of S/A ratio
- Abbott has indicated that will provide a minimum of 12 months notice before retiring the S/A ratio

What might this mean for labs?

- Refer specimens to a performing lab

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

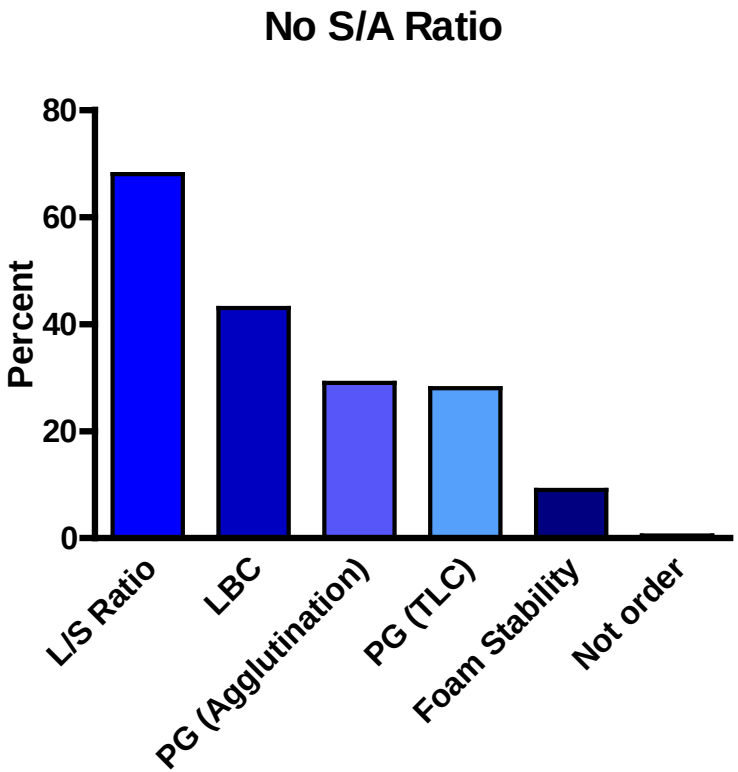


Unpublished data

What might this mean for labs?

- Provide an alternate FLM test

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



Unpublished data

Alternate Test Issues

- L/S ratio
  - Not recommended unless ≥15 specimens tested per week (Ashwood ER, *Clin Chem* 1997;43:211)
- PG
  - Late marker of maturity
  - Qualitative
  - History of reagent supply issues with manufacturer of agglutination test
- Foam stability
  - Lab developed test
  - Qualitative/semi-quantitative result
- LBC
  - Lab developed test
  - Quantitative result

THE LAMELLAR BODY COUNT

LBC Consensus Guidelines

Neerhof, at al. Obstet Gynecol 2001;97:318

- AF grossly contaminated with blood or meconium should be rejected
- AF should not be centrifuged (lowers the LBC)
- Cutoffs
  - Mature >50,000/µL
  - Intermediate 15-50,000/µL
  - Immature <15,000/µL

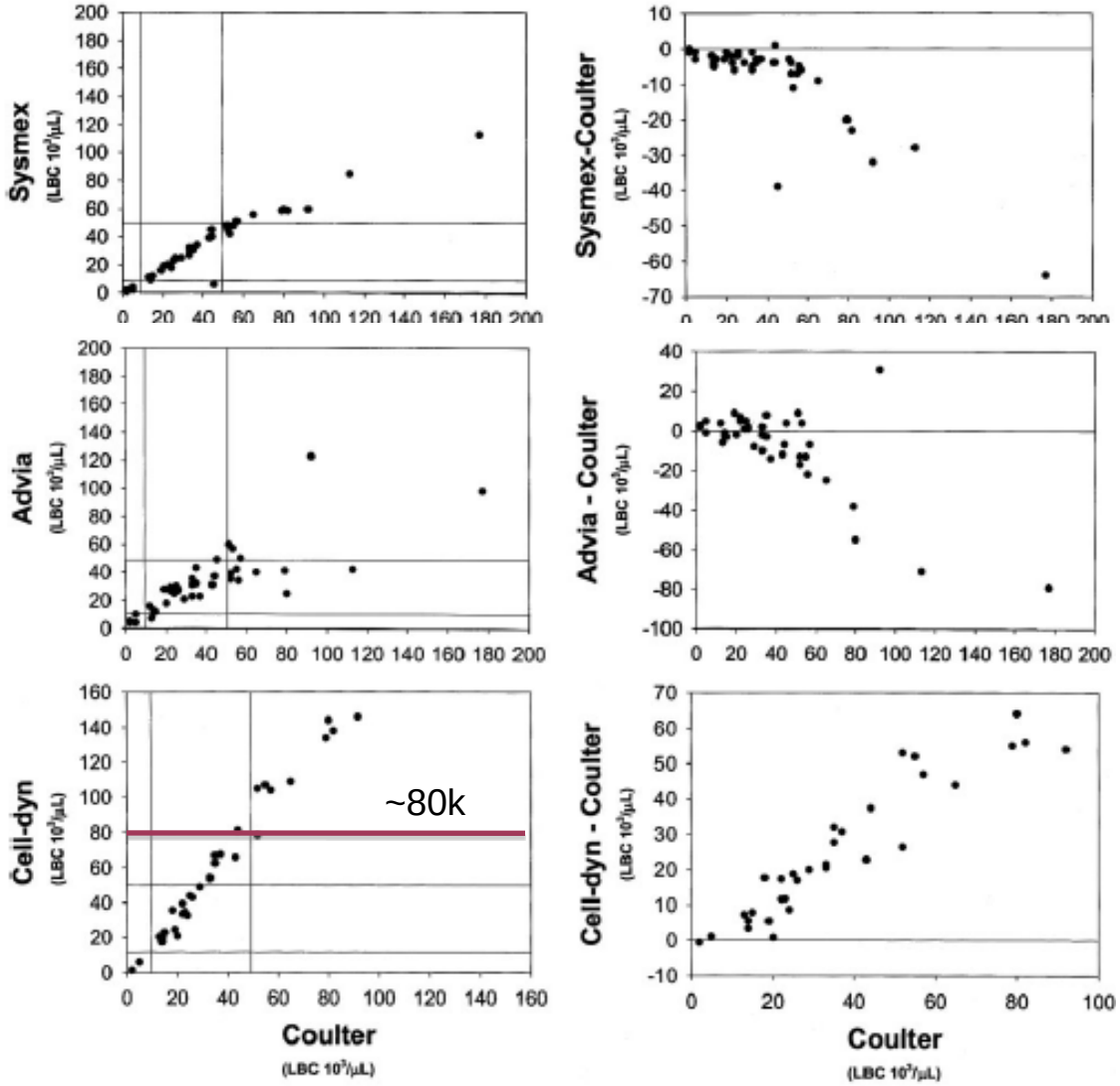
Did not address the impact of using different cell counters to quantify LBC

Cell Counters and LBC Cutoff

Sysmex

Advia

Cell-dyn



Concordance

36/42 (86%)

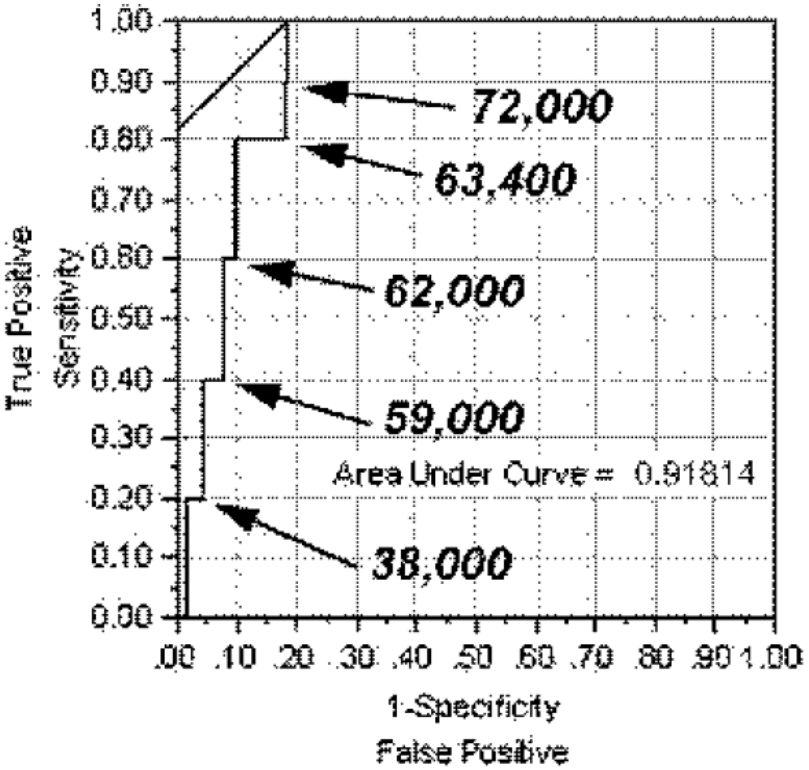
31/40 (78%)

21/32 (66%)

Clin Chem 2003;49:995-997

LBC on Cell-dyn

- N=209 (5 w/ RDS)
- LBC at GA of 35-38 weeks
- Cutoff of 72,000/µL was 100% sensitive

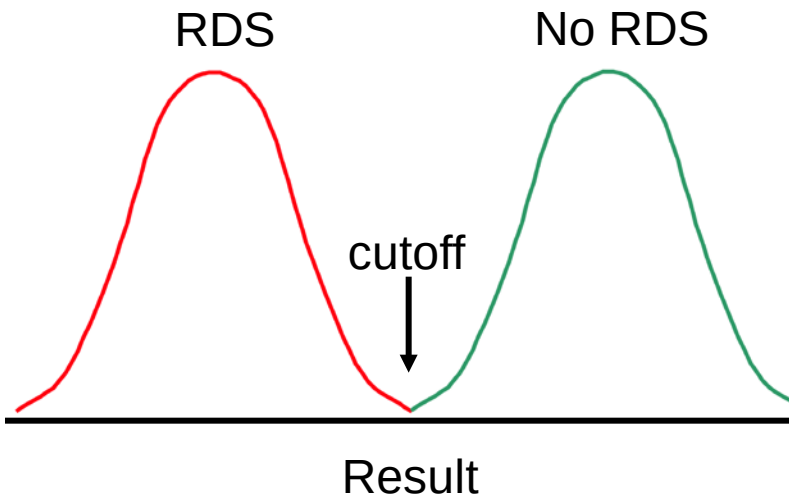


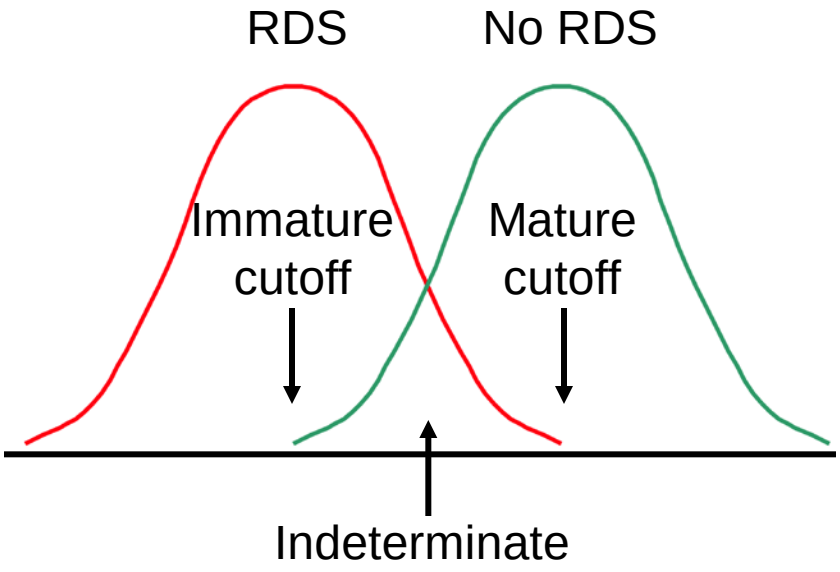
J Maternal-Fetal Neonatal Med 2009;22:419-422

Methods of Platelet Counting

Counter Brand (Manufacturer)	Platelet Counting Method
Coulter (Beckman)	Conventional impedance
Sysmex (Sysmex)	Conventional impedance
ADVIA (Siemens)	Optical light scatter
Cell-dyn (Abbott)	Conventional impedance & Optical light scatter

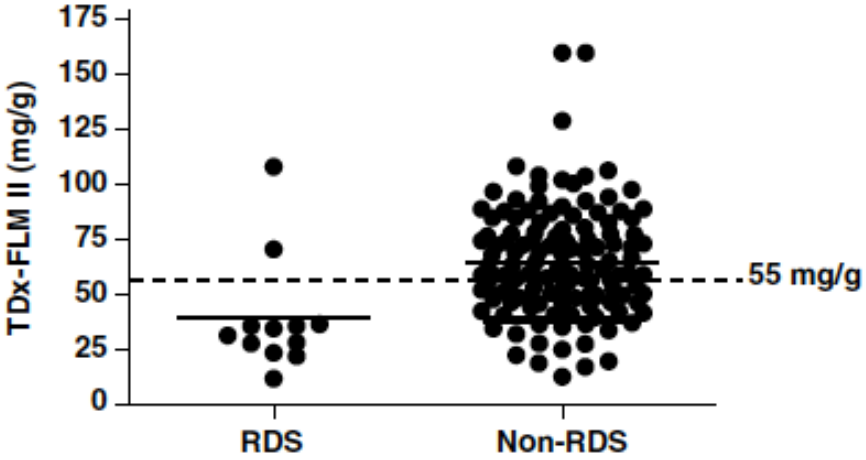
Cutoff Issues



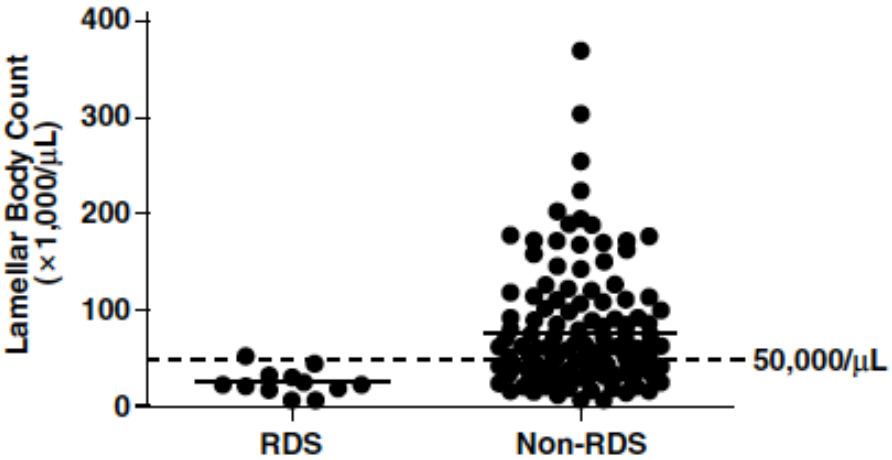


The Immature Cutoff

S/A Ratio

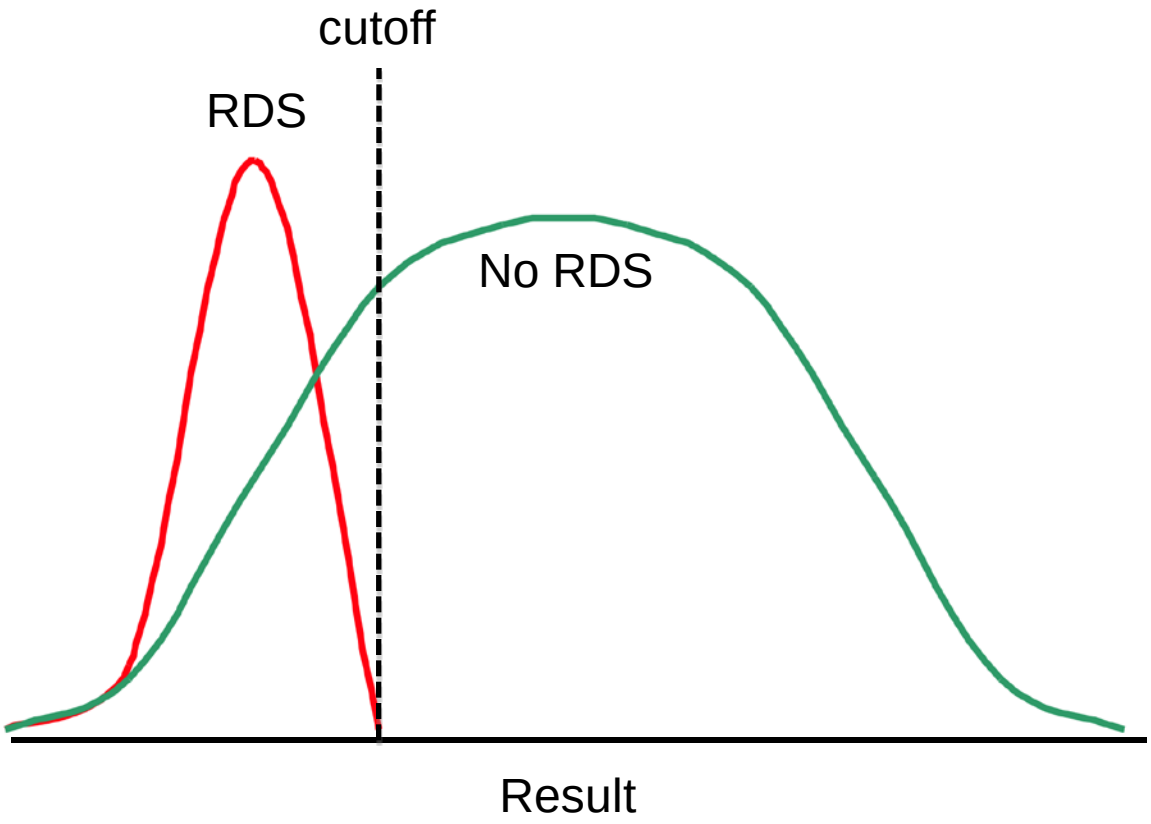


LBC

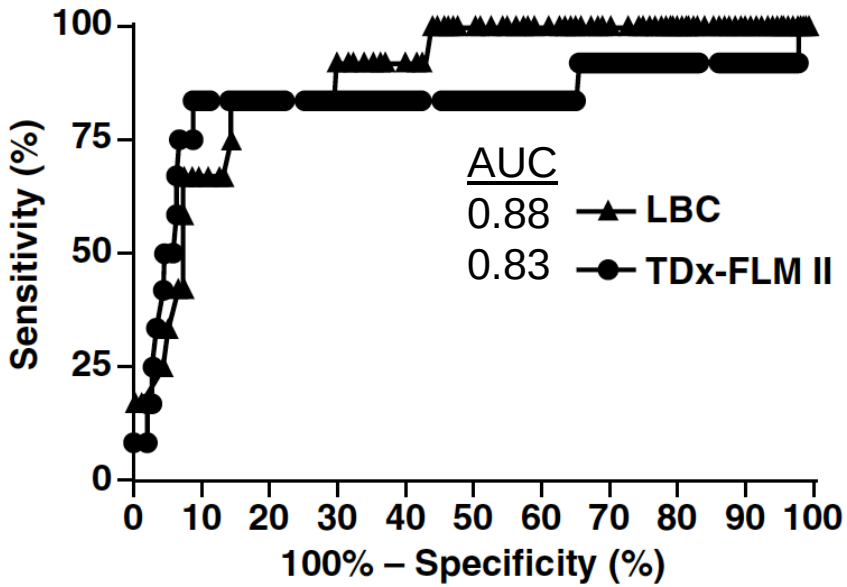


AJCP 2006;126:894-899

Cutoff Issues



LBC vs. S/A Ratio: Clinical Utility

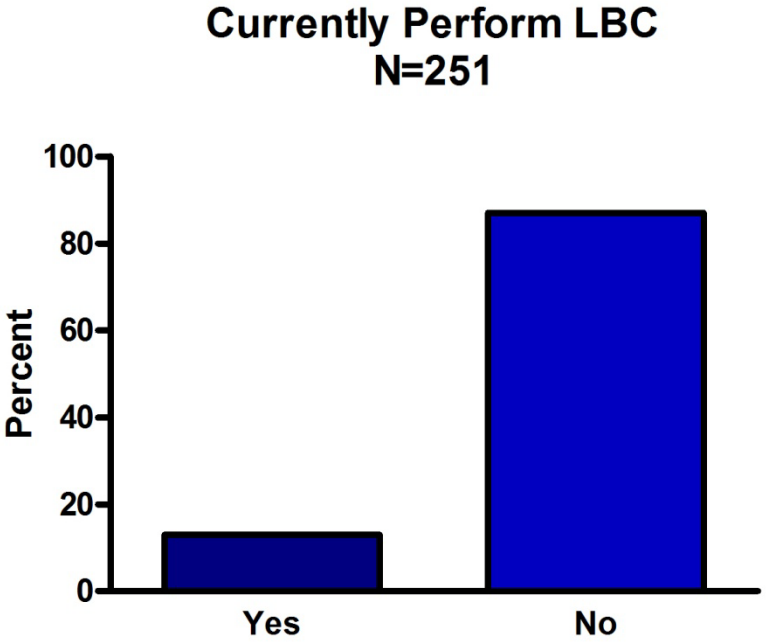


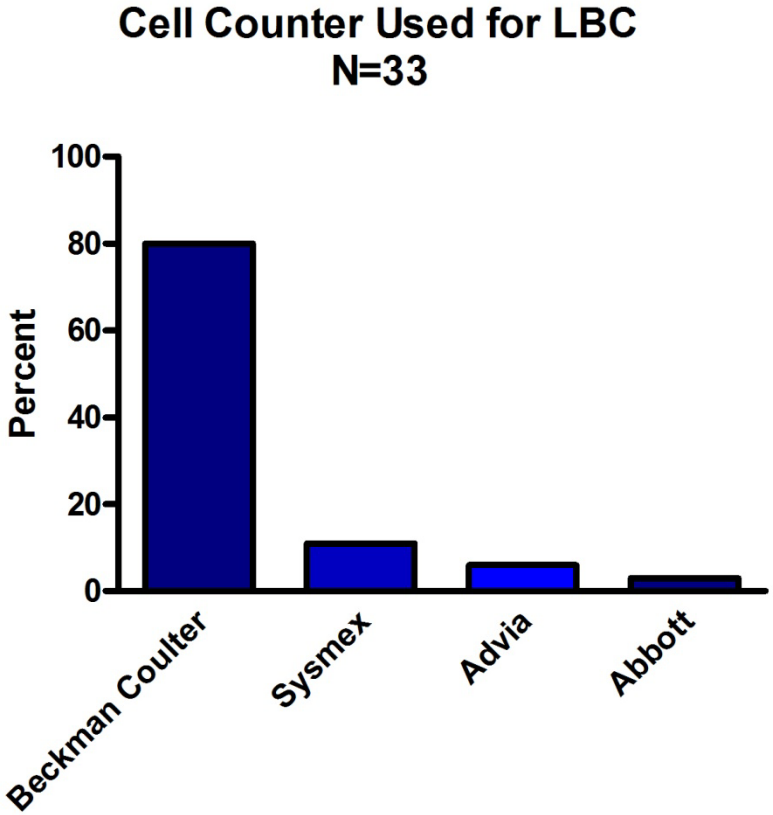
12 RDS
172 non-RDS

	Sensitivity	Specificity	Immature PV	Mature PV
LBC	92%	60%	14%	99%
TDx-FLM II	83%	65%	14%	98%

AJCP 2006;126:894-899

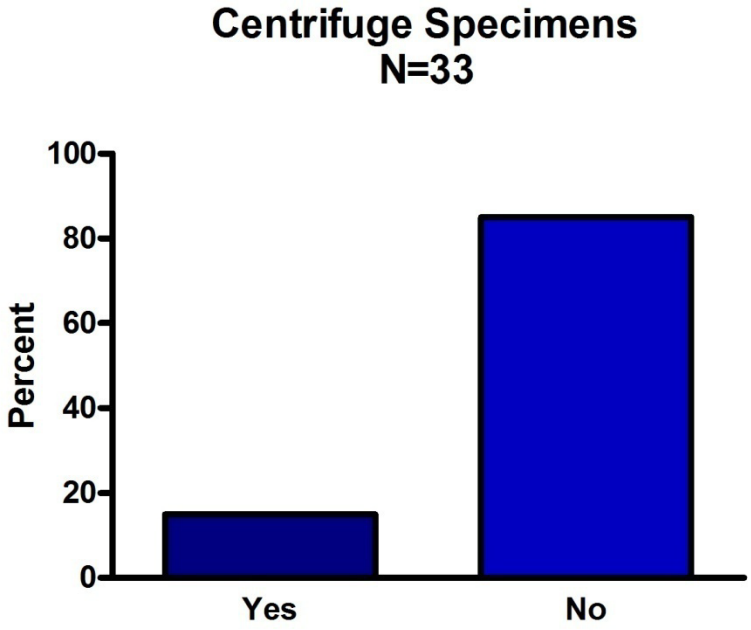
What are laboratories doing?

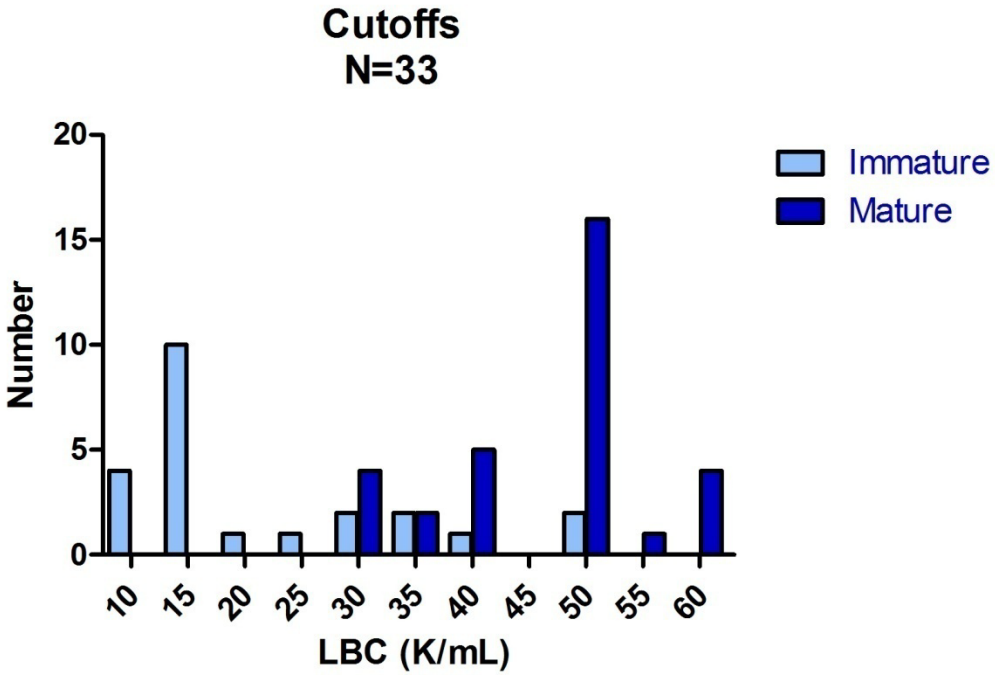




Unpublished data

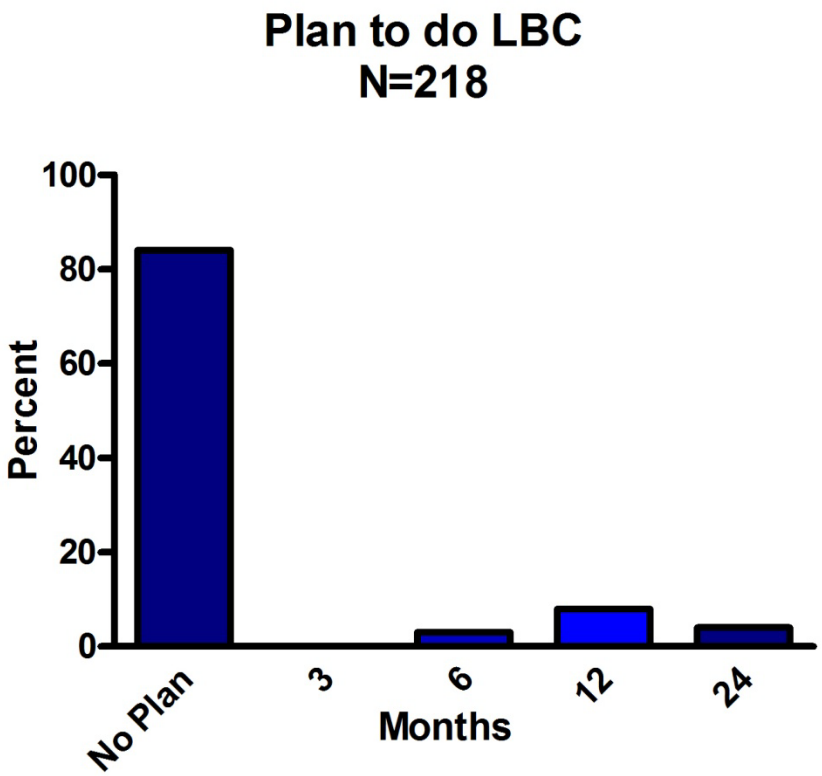
How are specimens processed and interpreted?

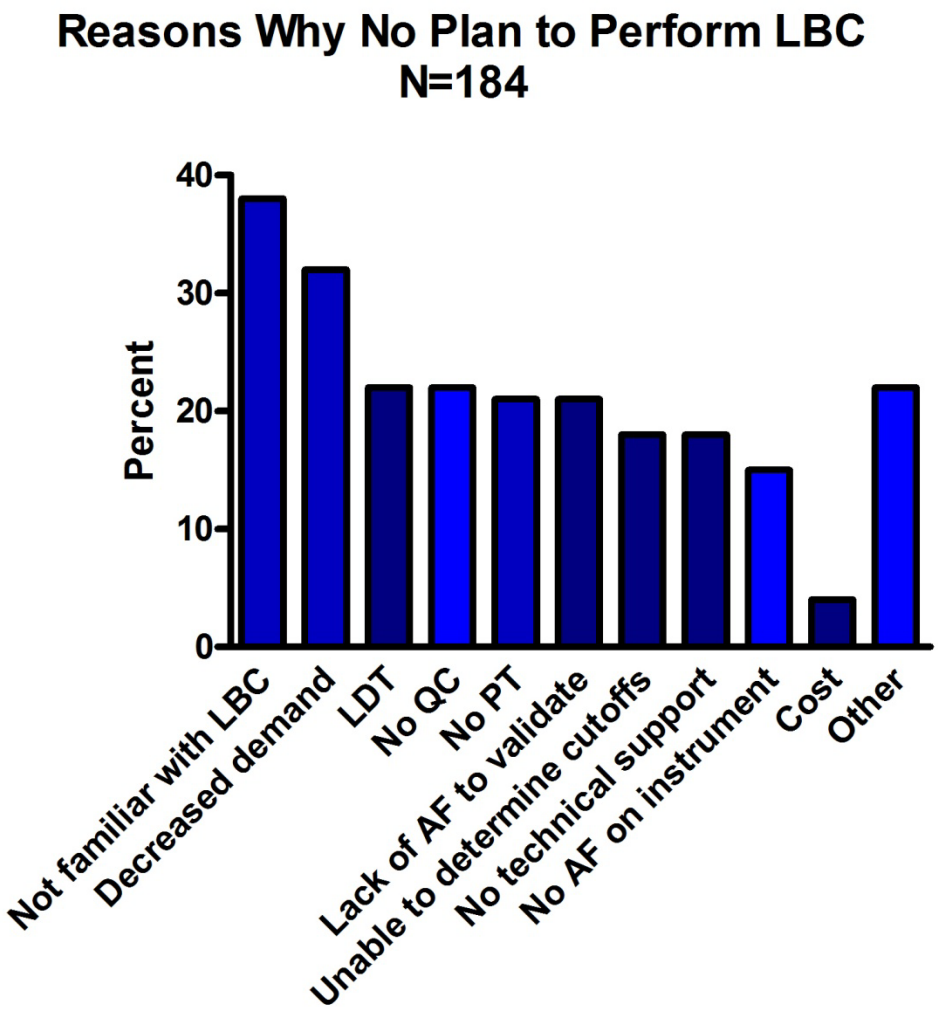




Unpublished data

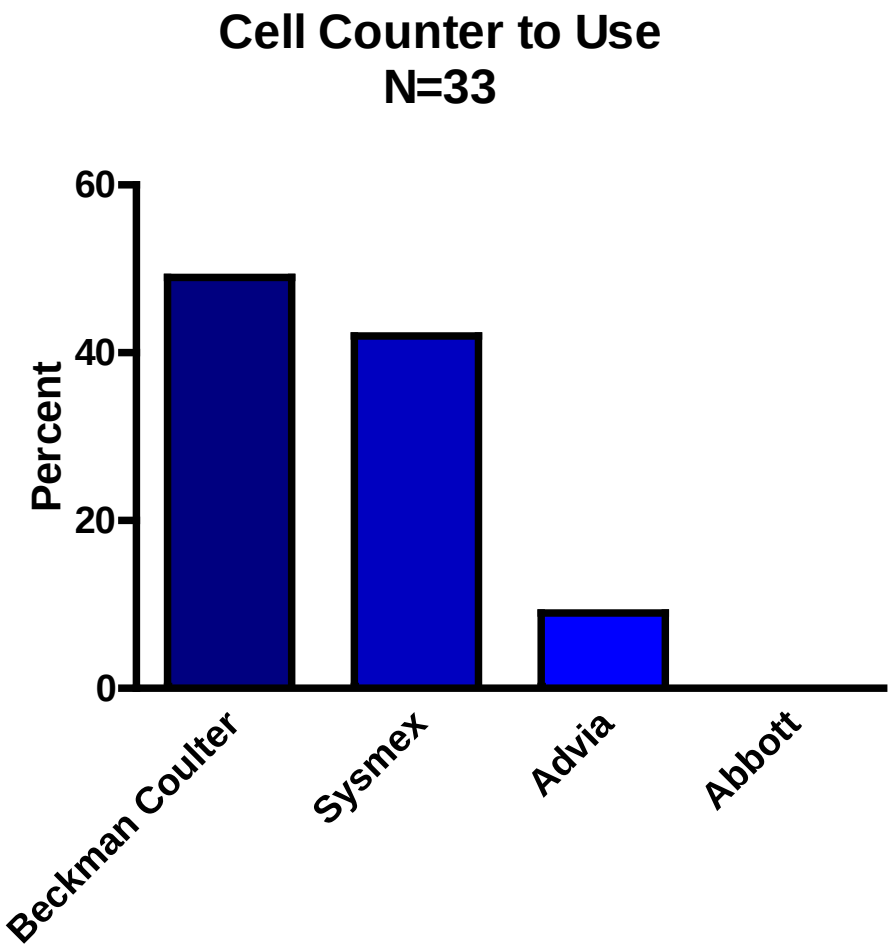
What do laboratories plan to do?





Unpublished data

What do laboratories plan to use?



Unpublished data

LBC Method Validation

- Protocol
- Imprecision
- Cutoff (reference interval)
- Analytical specificity
- Sample stability
- QC & proficiency testing

Protocol

Table 1. Protocol for Lamellar Body Counts

1. Mix the amniotic fluid sample by inverting the capped sample container five times.
2. Transfer the fluid to a clear test tube.
3. Inspect the specimen. Fluids containing obvious mucus or meconium should not be processed for a lamellar body count.
4. Place the test tube on a tube rocker for 2 min.
5. Flush the platelet channel; analyze the instrument's diluent buffer until zero is obtained in two consecutive analyses.
6. Process the specimen through the cell counter and record the platelet channel as the lamellar body count.
7. Notify the physician if the associated hematocrit exceeds 1%. The hematocrit is obtained from the hematocrit channel of the cell counter.

Obstet Gynecol 2001;97:318-320

Imprecision

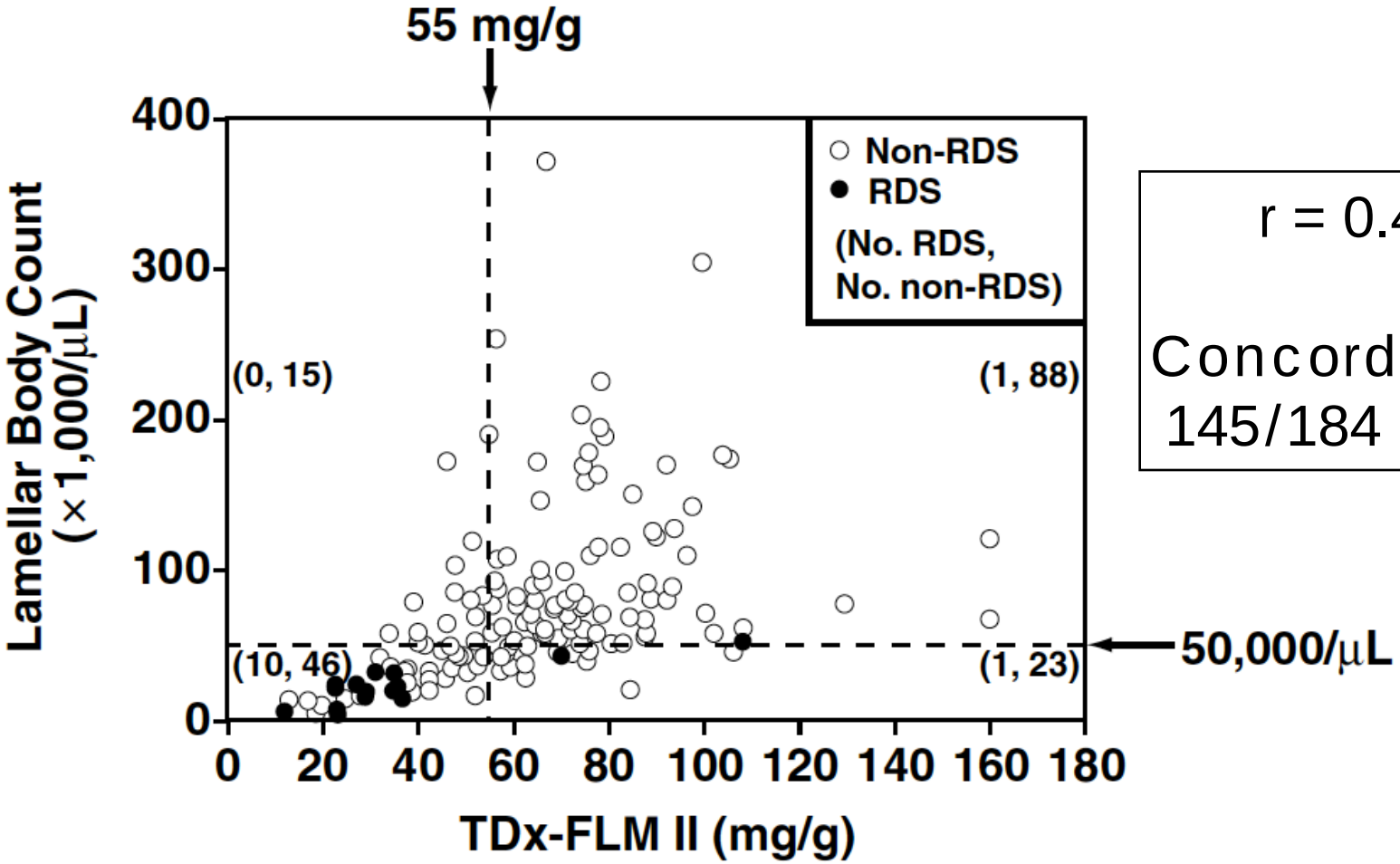
Counter	Mean	SD	CV
Coulter Gen-S	18,400	500	2.9%
Sysmex XE-2100	13,900	600	4.6%
Siemens ADVIA 120	12,965	855	6.6%
Abbott Cell-dyn 3500	31,300	2,100	6.7%

Clin Chem 2003;49:995-997

Establish/Verify Maturity Cutoff

1. Outcome based (gold standard)
2. Comparison with existing FDA-cleared method
3. Comparison with a well-validated LBC method

LBC vs. S/A Ratio: Comparison



r = 0.46

Concordance:
145/184 (79%)

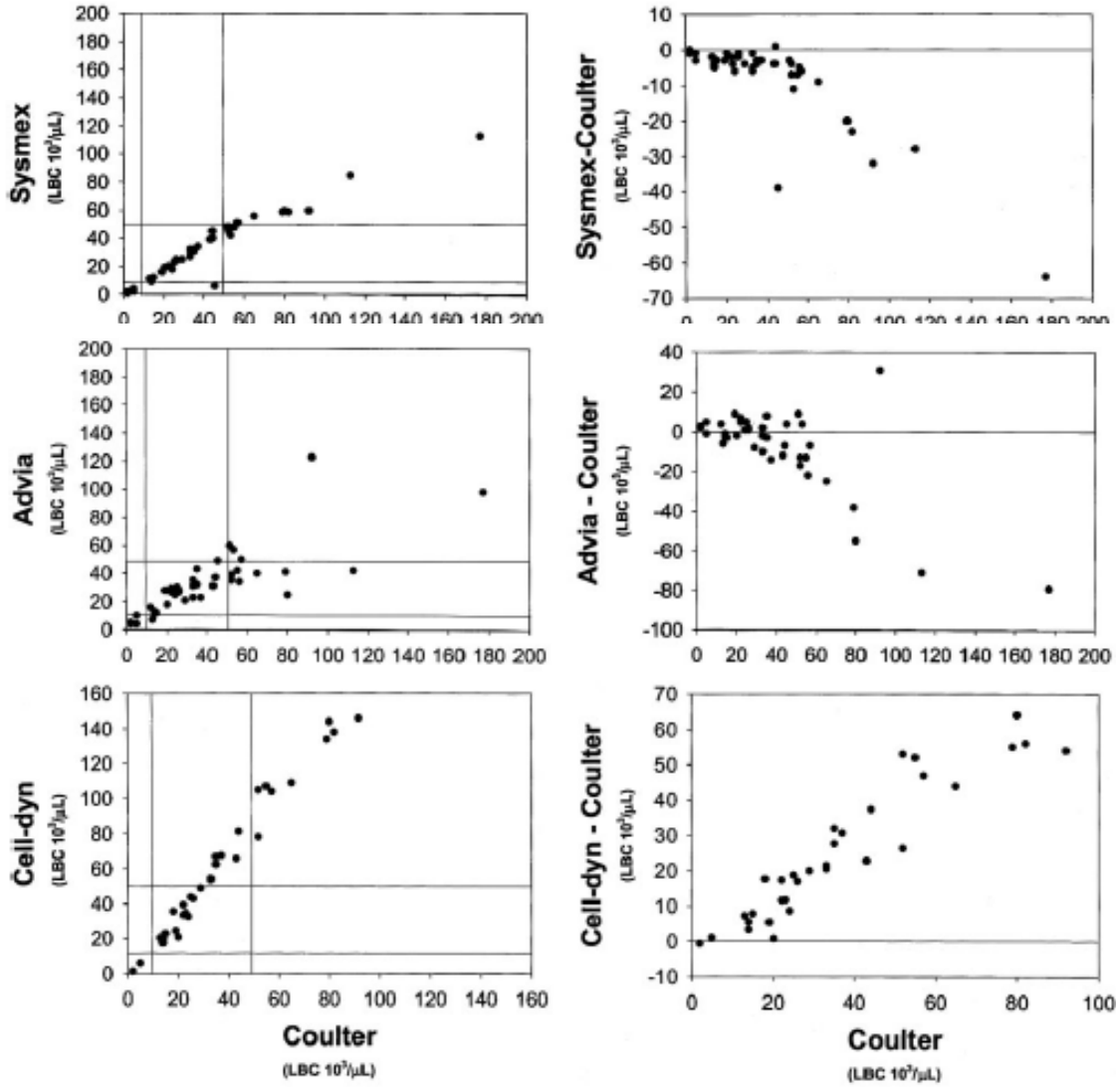
AJCP 2006;126:894-899

LBC vs. LBC: Comparison

Sysmex

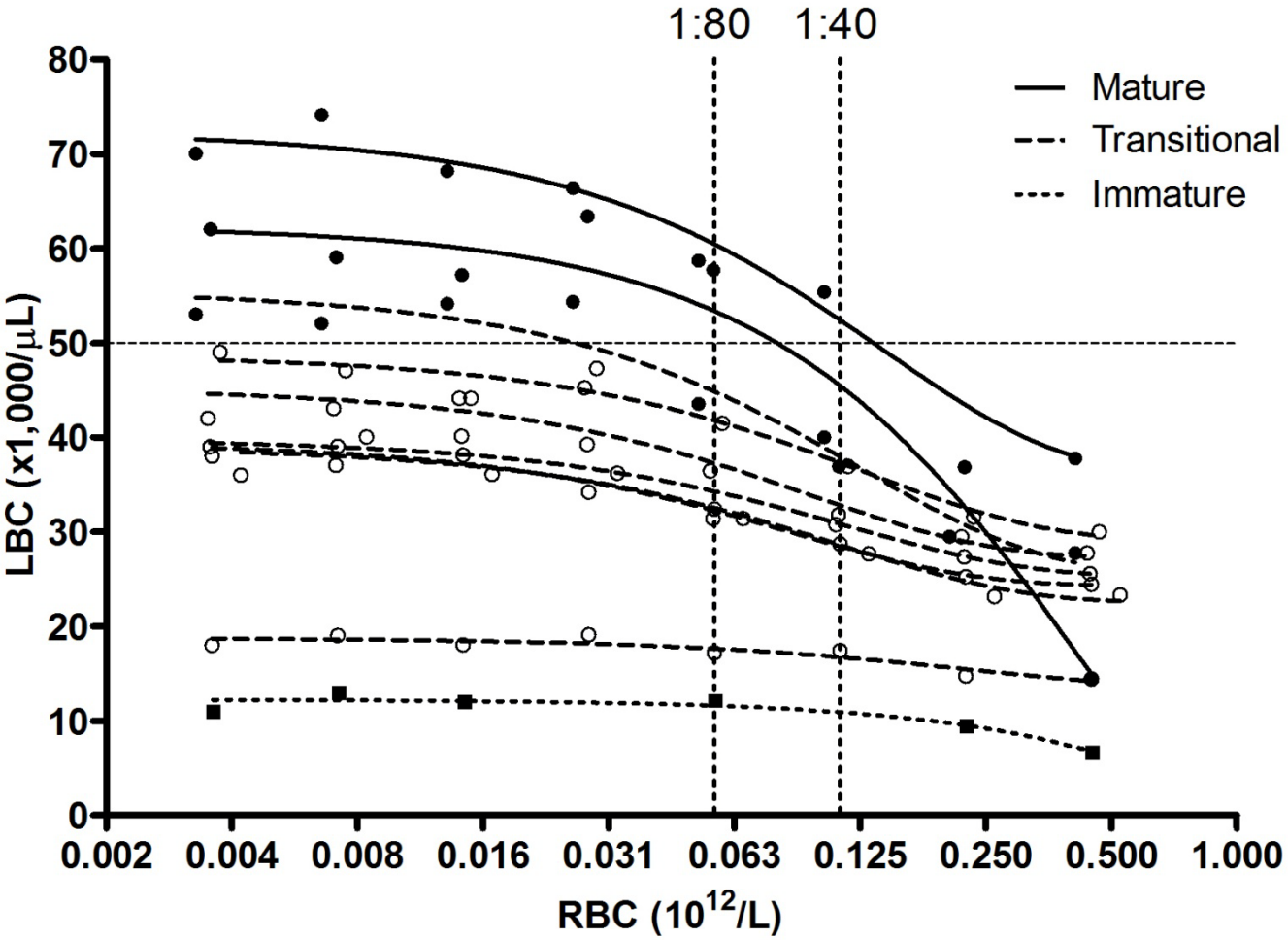
Advia

Cell-dyn



Clin Chem 2003;49:995-997

Analytical Specificity: Whole Blood



Unpublished data

Analytical Specificity: Whole Blood

RBC (10^3/µL): **4** **7** **15** **30** **60** **120** **240**

Analytical Specificity: Meconium

Unpublished data

Sample Stability: 4 C

Unpublished data