Detection Frequency by Thin-Layer Chromatography of Phosphatidylglycerol in Amniotic Fluid with Clinically Functional Pulmonary Surfactant

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Densitometric lecithin/sphingomyelin ratios (LSR) and the presence of phosphatidylglycerol (PG) were determined for 735 consecutively received amniotic fluids. Of the 371 fluids with "mature" LSR between 2.0 and 4.5, more than one-third lacked detectable PG. Clinical outcomes for the 305 of the total group that were delivered within 72 h of sampling were also determined. Respiratory distress syndrome (RDS) did not occur in the 239 cases with LSR ≥2.0, even when, as in 43 instances, PG was not detected. When the LSR was ≥2.0, transient tachypnea was more prevalent in the absence of detectable PG (PG detected, 3% transient tachypnea; PG undetected, 16% transient tachypnea). Of the 103 cases where PG was undetected, 58% exhibited no respiratory problems. Even in the 60 cases where the LSR was <2.0 and PG was not detected, 42% of the cases were free of respiratory problems. RDS did not occur in any case where PG was detected, even in the six where the LSR was <2.0. We evaluate these results in light of various contradictory reports in the literature.

Additional Keyphrases: lecithin/sphingomyelin ratio · fetal status · respiratory distress syndrome

Hallman et al. (1) incorporated identification of phosphatidylglycerol (PG) into the clinical procedure for amniotic fluid analysis in 1976. Results for 66 amniotic fluids from uncomplicated pregnancies formulated the basis for that report. PG was usually detected by the time the lecithin/sphingomyelin ratio (LSR) indicated maturity, always detected when the LSR exceeded 3.5, and was progressively more concentrated with increases in LSR. Predictive statistics on PG for normal pregnancies were reported by the same group in 1979 (2). They found that a third of the predictions of immaturity by LSR alone were incorrect. If PG was also looked for, the false-immature assessment rate declined to only 7%. Since then, different investigators assessed the degree of coincidence of PG with the LSR and also its clinical predictivity for fetal lung maturation. For unselected patient populations, the reported frequency of absence of PG with a mature LSR has varied widely, from 0 to 45% (3–7). Similarly, the reported frequencies of detection of PG with an immature LSR range from <1% to 75% (3–8). The frequency of occurrence of respiratory distress syndrome (RDS) when PG is absent was variously reported as 6% to 75% (3, 5, 7, 9). Thus, despite the widespread recognition of the association between amniotic fluid PG and fetal lung maturity, the frequency and predictive value of undetectable amounts of PG in amniotic fluid remain poorly defined.

We assessed the frequency of detection of PG over a range of LSR values by our previously published method in 735 consecutively received amniotic fluid samples (10). We then determined the predictive values of these measurements with this system, individually and in combination, for 305 cases delivered within 72 h of sampling. Because of the potential confusion caused by variations in analytical procedure between studies, we gave particular attention to correlation of our results with those previously obtained with other methodologies. To address the important question of the equivalence of the PG in amniotic fluid species detected here to the two-dimensional chromatographic method as performed by Hallman et al. (1), we chromatographed purified amniotic fluid PG and "pseudo PG" in two dimensions, and also used the unmodified "Helena Fetal-Tek" system of Touchstone et al. (11) and that of Robbins et al. (12).

Materials and Methods

Specimens of amniotic fluid were collected in the course of normal patient care and prepared and analyzed as described previously (10). All fluids were centrifuged at 1000 × g for 10 min before analysis or storage of the supernatant fluid at −20 °C. On thawing, the supernates were not recentrifuged; any particles that had formed in the freezing and thawing process were suspended evenly throughout the fluid before sampling for analysis. PG and "pseudo PG" were purified as follows: A pool of amniotic fluid that had been selected for large changes in apparent PG by chromatography (10) was first extracted and chromatographed in replicate lanes across a large chromatography plate (Whatman no. 4855-821, ASP cat. no. C5498-57; American Scientific Products, Houston, TX 77041) by the thin-layer chromatographic system of Touchstone et al. (11). The two outermost lanes, the first containing synthetic PG standard and the next the amniotic fluid pool, were broken off the plate and the phospholipids stained with cupric acetate reagent. This treated strip was then used to locate the region of the unstained plate containing PG and pseudo PG. The silica in that region was scraped from all the remaining lanes and extracted three times with a mixture of equal volumes of methanol and chloroform. That extract, containing PG and pseudo PG, was evaporated. The residue was dissolved in chloroform and chromatographed with our chromatography development sequence (10) in all lanes of another plate. Treating the marker lanes as described above guided us in scraping the strips of silica containing only PG or pseudo PG, respectively. The method of Touchstone et al. (11) (Helena "Fetal-Tek") was performed as described, as were the methods of Robbins et al. (12) and Body and Gray (13), as used by Hallman et al. (1).

RDS was defined clinically by rising oxygen requirements accompanied by the characteristic granular features of chest
roentgenograms (14). Cases in which sepsis was apparent or suspected were eliminated from the study because their pulmonary surfactant status could not be assessed reliably.

Results

LSR Correlation

Table 1 shows, for 735 consecutively analyzed amniotic fluids, the frequency with which PG was detected in amniotic fluid within discrete ranges of LSR. There is a clear lack of coincidence of the LSR threshold of maturity of 2.0 with the uniform detection of PG (PG/sphingomyelin ratio ≥ 0.05; actual PG concentration depends on degree of saturation). The point where PG is detected in 50% of the fluids occurs between LSR 2.5 and 3.0, and PG is not uniformly detected until the LSR exceeds 4.5, well beyond a reasonable LSR threshold value for maturity. This relationship predicts that many fluids in which PG cannot be detected will be associated with the absence of respiratory difficulty.

Clinical Outcomes

Table 2 shows the clinical outcomes of the 305 cases where delivery took place within 72 h of specimen collection and without sepsis. There was no respiratory difficulty in 42% of the cases where the LSR was <2.0; this is a significant but commonly reported proportion (2). We screened these cases to assess the possible role of steroid administration in creating this pattern [such therapy accelerates surfactant production without being reflected in the phospholipid pattern in the amniotic fluid (15)] and found that only nine of forty-five (20%) of the patients with LSR <2.0 but no RDS had been given glucocorticoids. We concluded that steroid administration was not a major factor in the false prediction of disease in this large group.

Detection of PG did effectively rule out RDS, but PG was detected only 9% of the time when the LSR was "immature." The false-immature prediction rate of the LSR value alone was decreased by additional consideration of PG, but only from 42% to 40%. There were no respiratory problems of any kind associated with 58% of the 103 fluids lacking PG or in 84% of the 43 PG-negative cases where the LSR was ≥2.0. Moreover, in the latter group, transient tachypnea was the only respiratory problem observed.

Table 1. Detection of PG as a Function of LSR

<table>
<thead>
<tr>
<th>LSR</th>
<th>n</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.4</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5–0.9</td>
<td>66</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0–1.4</td>
<td>106</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1.5–1.9</td>
<td>117</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>2.0–2.4</td>
<td>93</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td>2.5–2.9</td>
<td>87</td>
<td>47</td>
<td>54</td>
</tr>
<tr>
<td>3.0–3.4</td>
<td>77</td>
<td>58</td>
<td>75</td>
</tr>
<tr>
<td>3.5–3.9</td>
<td>61</td>
<td>54</td>
<td>88</td>
</tr>
<tr>
<td>4.0–4.4</td>
<td>53</td>
<td>47</td>
<td>88</td>
</tr>
<tr>
<td>4.5–4.9</td>
<td>28</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>5.0–5.9</td>
<td>22</td>
<td>20</td>
<td>91*</td>
</tr>
<tr>
<td>6.0–up</td>
<td>20</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>735</td>
<td>406</td>
<td>55</td>
</tr>
</tbody>
</table>

*Two cases in the 5.0–5.9 range for LSR lacked PG; one patient was from an outside hospital, and so records were not available; the second, who was not diabetic and did not have steroid therapy, delivered the next day with no respiratory problems in the infant.

Table 2. Outcome Frequency by Phospholipid Class for 305 Cases

<table>
<thead>
<tr>
<th>PG</th>
<th>LSR only</th>
<th>LSR and PG</th>
<th>PG only</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSR-only</td>
<td></td>
<td>LSR and PG</td>
<td></td>
</tr>
<tr>
<td>RDS = 0%</td>
<td></td>
<td>LSR+ and PG</td>
<td></td>
</tr>
<tr>
<td>TTN = 3%</td>
<td></td>
<td>RDS = 0%</td>
<td></td>
</tr>
<tr>
<td>OK = 97%</td>
<td></td>
<td>TTN = 4%</td>
<td></td>
</tr>
<tr>
<td>OK = 95%</td>
<td></td>
<td>OK = 96%</td>
<td></td>
</tr>
<tr>
<td>OK = 42%</td>
<td></td>
<td>OK = 58%</td>
<td></td>
</tr>
<tr>
<td>LSR- and PG+</td>
<td></td>
<td>RDS = 0%</td>
<td></td>
</tr>
<tr>
<td>RDS = 25%</td>
<td></td>
<td>TTN = 25%</td>
<td></td>
</tr>
<tr>
<td>OK = 35%</td>
<td></td>
<td>OK = 40%</td>
<td></td>
</tr>
</tbody>
</table>

In the study group where the LSR was ≥2.0, consideration of the presence or absence of PG did not improve the predictivity of the analysis for RDS. A slightly greater frequency of transient tachypnea occurred in this group where PG was not detected. Indeed, the only group seen here to benefit from the addition of the PG analysis is that small number of cases where the LSR was slightly <2.0 but detection of PG correctly predicted a mature outcome.

Thin-Layer Chromatography Validation

Because the fluids in which we did not detect PG represented a somewhat larger percentage than that reported originally by Hallman et al. (1) (see Discussion), we assessed the performance of the two chromatographic systems for identifying PG. The equivalence of the resolution of PG and pseudo PG by our analytical methodology and the two-dimensional thin-layer chromatography method originally used by Hallman et al. (1) is shown in Figures 1 and 2. PG and pseudo PG in amniotic fluid were purified for use in this analysis by sequential chromatography with two solvent systems: one in which they comigrate and one in which they are separated (see Materials and Methods).

Figure 1 shows resolution of purified PG and pseudo PG by the two-dimensional chromatography method of Body and Gray (13) as used by Hallman et al. (1). Figure 2 shows the chromatography of these same purified substances in three pertinent one-dimensional techniques, including that of Robbins et al. (12), which has been thought to resolve pseudo PG from PG (personal communication, Helena Laboratories). Of the three systems, only that using the prechromatography step separates PG and pseudo PG and can be expected to give results having significance similar to those obtained with the two-dimensional chromatography. The frequency of occurrence of pseudo PG in amniotic fluid has been described previously (10).

Phospholipids from 10 amniotic fluids with LSR >2.0 and undetectable PG (by our system) were chromatographed with the two-dimensional system. Figure 3 shows a representative analysis. In no case did analysis by two-dimensional chromatography show PG when it was not detected with our one-dimensional method.

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data suggest large differences in sensitivity of the methods for PG.

The prechromatography step we introduced in the thin-layer chromatography method to remove pseudo PG from PG could have decreased the sensitivity of detection of PG in our assay by removing a fraction of PG that was detected in the earlier reports based on two-dimensional thin-layer chromatography. The analytical data shown ensure that this is not the case; instead, the pseudo PG that comigrated with PG in the Helena method was also removed by two-dimensional chromatography.

Where PG is reported at very high frequency in the general population, the contradictory presence of PG when LSR is not mature is also frequent. For example, in reports (5, 6) in which PG was present in 89% and 93% of the overall fluids, PG was also detected in 51% and 72% of the cases where LSR was not mature. Conversely, when PG was detected at very low frequency, the occurrence of PG with immature LSR was also infrequent: Tsai et al. (8), for example, found that only 3% of fluids with LSR between 2.0 and 3.0 showed PG and, as expected, PG was detected in none of the fluids with immature LSR.

An especially high sensitivity for PG should increase the probability of RDS where PG was not detected, and this

**Table 3. Survey of PG Detection Data for Unselected Populations**

<table>
<thead>
<tr>
<th>Ref. no.</th>
<th>LSR+</th>
<th>LSR- 2-3</th>
<th>Total</th>
<th>PG- and LSR &lt; 2</th>
<th>All PG-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low sensitivity method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Our data</td>
<td>62</td>
<td>55</td>
<td>9</td>
<td>56</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>30</td>
<td>12</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>90</td>
<td>14</td>
<td>67</td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>50</td>
<td>44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Medium sensitivity method |
| 8       | 64   | 3        | 0     | 63              | 32     |

| High sensitivity method |
| 5       | 97   | 51       | 89    | 44              | 73     |
| 16      | —    | —        | 90    | 56              |        |
| 6       | 100  | 72       | 93    | 75              | 75     |
| 7       | 100  | 56       | 90    | 75              | 75     |

*In some cases data given for population subsets were recombined to represent the overall, unselected populations.

<sup>*</sup> = not determinable from published form of data.

<sup>**</sup>48% of this population had an LSR > 4, increasing overall %PG+.
relationship is, in fact, reported. The studies with very sensitive assays by Bent et al. (5), Kogon et al. (16), and Whittle et al. (6) (PG present in 89%, 90%, and 99% of the overall population, respectively) showed RDS at high overall frequencies when PG was undetectable: 73%, 56%, and 75%, respectively. In contrast, the apparently less sensitive assay of Tsai et al. (8) (3% PG present where LSR was 2.0–3.0) revealed only 12% RDS among those cases where PG was not detected.

Identifiable Methodological Biases between Studies

In some reports, additional undescribed biologic and methodologic variables may partly obscure the relationship between assay sensitivity and the occurrence of PG, but support for such a relationship comes from examination of procedural detail of the studies. Bent et al. (5) and Kogon et al. (16) analyzed amniotic fluid after centrifugally clearing the sample at the unusually low force of 140 × g for 5 min. They then recovered the lamellar-body pelleted obtained at high speed (10 000 × g, 20 min) from 10 mL of fluid and quantified the phospholipids by phosphate assay after chromatography. The combination of low clearing speed, large fluid volume, and sensitive chemical detection of phosphorus would be expected to make their assay more sensitive than that of Tsai et al. (8). Tsai et al. analyzed PG recovered from a smaller volume (3 mL) of amniotic fluid after clearing at 1000 × g for 5 min, conditions that Oulton found (17) would include some of the surfactant phospholipids in the pellet. They then washed the samples for 30 min with acetone, in which phospholipids could be lost, then used acid charring with sulfuric acid/water (equal volumes) at 300 °C, which may allow partial loss of lipid by volatilization (18).

There is less reason to predict that the methodology of Whittle et al. (6) would be especially sensitive for PG, as we postulated from studying their data. Like Tsai et al. (8), they cleared the amniotic fluid at higher speed (1100 × g, 5 min) and used both acetone precipitation and lipid charring. However, they channed with ammonium sulfate rather than sulfuric acid, at a lower temperature (250 °C), and quantified by planimetry rather than densitometry. All these differences substantially alter quantitative phospholipid results (19,20) and may be important in the high sensitivity of the latter workers' assays.

Other Potential Methodological Influences in Detection of PG

Some aspects of the procedure that greatly influence the final results and may be important in differences between studies are often not described in detail: conditions of temperature and time for the precipitation or wash of the dried phospholipids with acetone, a step not used in the initial report on PG in amniotic fluid (1) but implemented in the later study (2). Other analytical details usually not included in these reports but which may be important to sensitivity for detecting PG include the performance of the densitometer as influenced by beam width relative to spot width, the rate at which the plates are brought to the final temperature (21), and the inclusion or exclusion of a centrifugation step for the specimens after freezing and thawing.

Sulfuric acid, ammonium sulfate, and cupric acetate, as detection reagents, have well-documented differences in sensitivity for various degrees of saturation of the fatty acid side-chains of the phospholipids (11,22,23). This variation makes the definition of sensitivity for PG by thin-layer chromatography meaningless unless one uses pure phospholipids whose saturation is equivalent to that of the pulmonary surfactant phospholipids, which are 60% fully saturated and 38% singly unsaturated (24).

Methods also vary with regard to specific interferences. Although some (24–26) have reported that meconium does not interfere in their methods for determining PG, a color-reactive substance in meconium that could be mistaken for PG has been recognized in our system (data not shown). This band, more diffuse than PG, is usually associated with visibly green-tinged fluids, but it presents a potential for false identification of PG. In some systems, components in blood may also mimic PG (27), although in our system and others (28) this is not the case.

Variation in results in different studies may be introduced by important differences in specimen handling that influence the actual concentration of PG, independent of analytical methodology. The particulate nature of the pulmonary surfactant complexes that carry PG (29), balanced against the need to remove cellular and particulate interferents, has long been recognized as a complication in the analysis (17) and is a potential source of variability between laboratories for PG methods that involve centrifugation.

The Potential for Biological Bias among Study Populations

Differences in the frequency of detection of PG in maternal disease states have been reported (30), and differences in patient populations could confuse interstudy data. In 1978 Cunningham et al. (31) first reported the delayed appearance of PG in amniotic fluid from diabetic mothers. However, that study appears in retrospect not to have been actually measuring PG, because the highest "PG" concentrations were associated with gestations of <33 weeks, decreasing to the lowest values beyond 38 weeks (31).

In 1979, Bustos et al. (32) concluded from studies of seven diabetic patients that PG production was accelerated relative to gestational age in diabetics in classes D, F, and R. A second report (33) by that group indicated that diabetics of classes B, C, D, F, and R (n = 177) were indistinguishable from normals but, rather, that PG was delayed in class-A diabetics (n = 43). A third report (34) described delays in PG production in all classes of diabetics, especially B, C, and D (n = 74), with LSR used as an index of timing. The trend of delayed production of PG relative to LSR in diabetics was reported more recently as well (8,25), but others failed to confirm differences in timing of its appearance in diabetics (35). In contrast to the widely cited findings of Robert et al. (36), the increased frequency of RDS for diabetic populations has not been uniformly observed (37). This failure to reproduce previously documented phenomena may reflect improved clinical management of diabetics (37). Alternatively, other biological variations in the patient populations—gestational age distribution, gender (38), or stressful conditions such as maternal hypertension (30)—may have contributed to the inability of various workers to unequivocally define a consistent influence of diabetes on the development of PG.

Of the 43 predictive cases in our study with a mature LSR but no PG, only 9% were diabetic; thus our observation is clearly not ascribable to delayed PG production by infants of diabetic mothers. We have not separated our diabetic patients for probability calculations because of the questionable basis in the literature for making such a difference and the relatively small size and low statistical power in our population.
The Importance of Sensitivity or Definition of the PG Signal

Definition of a "positive" PG has varied among studies and represents another major procedure-dependent aspect of the detection of PG. The original expression of PG in amniotic fluid was as a percentage of the total lipid phosphorus determined by measuring and summing all the major phospholipids after chromatography (1). A transition to the more convenient approach of quantification from densitometry, where the detection method is not phosphate-specific, required summation of the lipid signals for the known phospholipids but still allowed expression of PG as a proportion of the total major phospholipids (2). Remarkably, given that the two assay principles are totally different, the final percentages were nearly equivalent, and in one report, they were averaged together for the final result (34). The percentage scored as PG-positive by this approach is usually 2–3% of the total lipids or phospholipids, depending on the detection method (2, 8). To avoid the analytical uncertainty introduced by identifying and quantifying five distinct species of phospholipids simply to represent the concentration of PG, we chose to represent the data in a different form. PG was expressed here as a densitometric ratio to sphingomyelin, analogous to the LSR, as was done by others (6, 39, 40, 41). We considered PG "positive" when the PG/sphingomyelin ratio was >0.05, which was chosen because it is practically equivalent to the point at which a PG band can be seen on the thin-layer chromatography plate.

That the "appearance" of PG by these methods is equivalent to the onset of pulmonary maturation is challenged by data from highly sensitive enzymatic methods. Such data replace the older concept with a pattern of low concentrations of PG being present in amniotic fluid long before maturity, but increasing rapidly at the time the amount of surfactant becomes adequate (see below). That perspective would account for the variable and method-dependent data on the "presence" of PG reported with thin-layer chromatography. Further, one might therefore predict that if the sensitivity (detection limit) of a given method were the same as the concentration of PG that corresponds to lung maturity, this would simply be a coincidence.

Current Perspective of the "Presence" of PG

Thin-layer chromatographic methods with staining or charring and quantification by densitometry or planimetry vary in their sensitivities for PG and other phospholipids and give a wide range of results. Therefore, they cannot provide reference data to define the onset of PG production. The most sensitive and specific thin-layer chromatographic assay of PG has involved analysis for organic phosphate after recovery and extraction with organic solvents of the phospholipid species from the chromatography plate. So measured, as little as 0.5 mg PG per liter can be detected in amniotic fluid (16). This method eliminates dependence on the degree of side-chain saturation seen with thin-layer chromatography staining or charring and also avoids interference by the many components of amniotic fluid that react with the visualization methods but do not contain phosphorus. The limitation of the phosphorus analysis method is that one may not totally recover one single chemical species (spot) from the plate without including other compounds from the background, when a visual detection method that is not itself sensitive to phosphorus is used as the guide.

Recently developed enzymatic assays for PG in amniotic fluid offer a potentially sensitive and specific reference method for the uniform study of this analyte. The assay of Farquharson et al. (42), designed to rigorously remove endogenous glycerol, subjects the standards to the steps of analysis in which significant losses were shown to occur. The sensitive two-dimensional thin-layer chromatographic method of Whittle et al. (6) (Table 3) used in the evaluation of this enzymatic method was negative for PG in every case in which PG was also undetected by the enzymatic assay. On the other hand, the enzymatic assay detected PG in more than half the samples in which thin-layer chromatography failed to detect it, but at concentrations generally <1 mg/L (expressed in terms of dipalmityl PG). In almost all cases, values for PG were >1 mg/L by enzymatic assay when the thin-layer chromatography result was positive. These data indicate that PG is indeed present in amniotic fluid at a time when, with traditional assays, it was regarded as "absent."

Concerns over the specificity of different enzymatic methods have been raised, particularly in regard to assays that indicate several-fold this much PG at all times through gestation (43, 44). A recent report (45) showed that the glycerol concentration in amniotic fluid may be 10- to 20-fold as great as that of PG, and may well be responsible for the high concentrations of PG in amniotic fluid reported in some early studies. This conclusion would favor the lower PG concentrations reported by Farquharson et al. (42), after rigorous removal of glycerol. The latter workers reported the presence of PG very early in gestation but at concentrations too low to be detected by thin-layer chromatographic methods.

The clinical significance of the qualitative detection of PG by sensitive methods such as enzymatic assays or immunoassay (46) will depend on the particular assay's detection limits, just as it does with the various thin-layer chromatographic assays. Use of quantitative methods, in contrast, will allow concentrations of PG to be determined that actually correspond to the onset of fetal lung maturity.

Conclusion

Our data, considered together with previous reports and the known sources of variability in PG determination, make it clear that current routine methods for detecting PG in amniotic fluid by thin-layer chromatography cannot be considered absolute or equivalent. Data collected by thin-layer chromatographic methods, designed primarily to be practical in the clinical laboratory, should be viewed not as research data for defining the correlation of amniotic fluid phospholipids with clinical outcomes. Rather, such data constitute the controlled database needed to define the specific performance of a thin-layer chromatographic system in predicting disease.

The potential for confusion from generalizing data on the "presence" of PG as if it were method-independent (e.g., ref. 47) is apparent. Many thin-layer chromatographic methods for PG have been published for clinical use (1, 10, 25, 39, 40, 48, 49). The College of American Pathologists' proficiency survey regularly shows that ~5% of the participants report PG when it is not present, or report its absence when it is in fact present—further evidence of the significance of the heterogeneity of methods for PG in use in clinical laboratories today.

We think that simultaneous determination of the LSR and PG remains the soundest means of evaluating fetal pulmonary maturity, by providing two independent pieces of information whose extreme inconsistency may reflect analytical interferences. In such a case, the presence
meconium (see above), bacterial colonization as a source of PG (50), or interference with the LSR by blood (51) can be considered as possible causes of variance, allowing one result or the other to be considered the more reliable.

Our data define the predictive response of this analytical system in a group of unselected cases. From these results, we recommend a primary reliance on the LSR with this system, except when an immature LSR is contradicted by the detection of PG; in such cases, the PG, given the threshold of sensitivity of this assay, predicts fetal lung maturity reliably.

References
42. Farquharson J, Jamieson EC, Paton RD, Black J, Logan RW.

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