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A Cost-Effective Modification of an Immunologic Agglutination Test for Amniotic Fluid Phosphatidylglycerol

To the Editor:

The appearance of phosphatidylglycerol (PG) in amniotic fluid marks one of the final stages of lung maturation. Consequently it is unusual for a neonate to develop hyaline membrane disease once a significant concentration of PG has appeared in the amniotic fluid. However, many neonates do not develop hyaline membrane disease in the absence of a significant concentration of PG in amniotic fluid (1). The lecithin/sphingomyelin (L/S) ratio is the primary laboratory test used to assess fetal lung maturity. The lung usually becomes mature one to two weeks before PG can be detected. Some clinical conditions such as diabetes, intrauterine retardation, and contamination of amniotic fluid by blood or meconium do give false-positive L/S ratios. It is in these situations of that the PG test is most helpful.

Phosphatidylglycerol has been commonly measured by chromatographic methods that are based upon the work of Gluck et al. (2). Recently, two quantitative chemical methods for measuring PG have been described (3, 4), as well as a qualitative slide agglutination test marketed under the name AmnioStat-FLMTM by Hanna Biologics, Inc., Berkeley, CA 94710. The quantitative chemical methods both suggest that PG concentrations exceeding about 5 μmol/L are associated with fetal lung maturity. The qualitative test is compared with a positive control set at about 3 μmol/L. Two clinical studies (5, 6) evaluated the qualitative test and reported that positive results were associated with the absence of hyaline membrane disease while less than half the babies with negative tests developed the syndrome. Consequently, in the presence of a positive test, no other test was required; but when the results were negative, other tests such as the L/S ratio or fluororescence polarization were used to confirm lung maturity.

Measurement of PG is expensive, either because of technically involved methodology or because of costly reagents. We have been successful in reducing the amount of reagents required for the qualitative test to less than one-tenth, while maintaining essentially the same procedural steps.

All reagents were provided in the AmnioStat-FLMTM kit obtained from Hanna Biologics, Inc. The reagents consisted of (a) a negative control; (b) a positive control, containing about 3 μmol of PG per liter; (c) reagent A, consisting of a solution of lecithins and cholesterol in alcohol; (d) reagent B, containing antibodies to PG; and (e) a phosphate dilution buffer. All reagents were dispensed with Oxford micro pipets or Oxford Ultra-Micro SamplersTM (Lancer, St. Louis, MO 63103). The Ultra-Micro Samplers are accurate to within ±2% and CVs = 1.0% at 1 or 2 μL. The reagents were stored at 4 °C, but were brought to room temperature before use. The reactions took place in the wells of a Teraseki tissue-typing tray (Robbins Scientific Corp., Mountain View, CA 94043). These trays contain 72 wells, each of which can hold 12 μL of solution. The wells have the shape of an inverted, truncated cone, with the walls sloping down to an optically clear, circular area about 1.0 mm in diameter. A pair of wells were used for each sample or control.

Amniotic fluid specimens were centrifuged at 500 × g for 3 min to remove cells and debris. One microliter of each control or specimen was pipetted into the bottom of a designated well and covered with a glass cover slip to minimize evaporation. One microliter of reagent A was then thoroughly mixed with each specimen by drawing the mixture into the pipet tip and re-dispersing several times. Next, 10 μL of the phosphate dilution buffer was added and thoroughly mixed. Two microliters of reagent B was placed in the second of each pair of wells, followed by 1 μL of suspension from the first well. The solutions were thoroughly mixed, a cover slip was placed over the wells, and the tray was set aside. After 1 h the contents of each test well were gently mixed by using a micro pipet. Then the cover slip was replaced and the tray was set aside. The patients' specimens were microscopically determined to be positive or negative by comparison with the controls, with 100× magnification. Figure
Fig. 1. Phosphatidylglycerol agglutination reaction under 100× magnification
Left: no agglutination, negative reaction. Right, agglutination with clearing of the background, positive reaction

1 shows a negative control and a positive result.

It is essential that reagent A be thoroughly mixed with each specimen. This causes the cholesterol and lecithins from reagent A to come out of solution, forming a fine, needle-like suspension with PG, if present. When reagent B is added, the particles will agglutinate only when the suspension contains PG. The final mixing before reading is necessary because the suspension settles out on the sloping walls of the reaction wells. If mixing is too vigorous, the aggregates can be broken up, giving the appearance of a negative reaction. For this reason, it is wise to perform the assay in duplicate, particularly when it is being performed by inexperienced personnel.

The only significant modification we have made in the use of the AmnioStat-FLM kit is to scale down the use of reagents. Consequently we expect our procedure to yield equivalent results. Our limited clinical experience has so far supported this assumption. None of 63 neonates delivered after a positive, modified AmnioStat-FLM test showed any evidence of hyaline membrane disease. One of five babies delivered after a positive L/S ratio but negative, modified AmnioStat-FLM test did develop limited hyaline membrane disease. Ten specimens, run by two-dimensional chromatography, yielded seven positive PG results and complete agreement with the modified AmnioStat-FLM test. We also found exact concordance when the AmnioStat-

FLM kit was run by the standard procedure and by our modified protocol.

References


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Ed. note: See also the Note on pp 1233–
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