Coulter STKS vs Abbott Cell-Dyn 3500 for Counting Lamellar Bodies in Amniotic Fluid

ABSTRACT Lamellar body counts in human amniotic fluid assist in the prediction of fetal lung maturity and can be performed rapidly and inexpensively by using the platelet channel of a hematology analyzer. We compared two analyzers for enumerating lamellar bodies. We tested 100 fresh amniotic fluid samples on both instruments. The lamellar body counts ranged from 3 to 364 \( \times 10^3/\mu L \) (3–364 \( \times 10^9/L \)) on one analyzer and from 3 to 195 \( \times 10^3/\mu L \) (3–195 \( \times 10^9/L \)) on the other. Statistical analysis showed excellent correlation \((r = 0.99)\) yielding a regression equation of \( y = 3.91 + 0.467x \). This analysis demonstrates that institutions will be faced with the challenge of establishing and incorporating instrument-specific lamellar body reporting protocols and reference ranges that include the specific analyzer and method used.

Fetal lung maturation is a complex process of events that involves a balance of physiologic and cellular biologic functions and lung development. The lungs are one of the last major organs to mature in the fetus. Prenatal analysis of amniotic fluid for fetal lung maturity is instrumental in the clinical management of maternity patients. Various laboratory biochemical and biophysical tests can help determine lung maturity when obstetric complications arise, such as preeclampsia or preterm labor, and delivery is imminent. The tests rely on the premise that the amniotic fluid composition accurately reflects the maturation of the fetal lung. Characteristics of routinely ordered tests to assess fetal lung maturity are shown in Table 1. The lamellar body count is a rapid test that can be used to help the clinician make this determination.

Lamellar bodies, concentrically layered structures of protein and surfactant associated with pulmonary maturity, are secreted by type II fetal pneumocytes. The surfactant contained in the lamellar bodies decreases the surface tension in the fetal lungs and prevents them from collapsing after delivery. As fetal lungs mature, lamellar bodies increase in number and are expelled into the amniotic fluid via fetal breathing movements. Their presence often gives the amniotic fluid an opalescent appearance. Neonates who are unable to synthesize adequate amounts of surfactant can develop respiratory distress syndrome, a serious life-threatening condition (Fig 1). This syndrome may require intratracheal administration of exogenous surfactant, as well as supplemental oxygen therapy, resulting in an extended hospital stay for the neonate.

Enumerating lamellar bodies by using the platelet channel of a commercial hematology analyzer can be readily accomplished owing to the availability of these instruments in most laboratories. Because their size is similar to that of platelets, lamellar bodies are recognized and counted through the platelet aperture.

Amniotic fluid requirements are minimal, and the results can be available in less than 10 minutes. A strategy to optimize laboratory testing capabilities, using the most reliable and efficient tests first, can assist the physician in making a clinical decision should rapid intervention be required in the pregnancy.

Use of the Coulter whole blood analyzer (Coulter Electronics, Hialeah, Fla) to count lamellar bodies has been described. Our objective was to compare the Coulter STKS and the Abbott Cell Dyn 3500 analyzer (Abbott Diagnostics, Abbott Park, Ill) for counting lamellar bodies in amniotic fluid. The lamellar body counts were further evaluated in relation to neonatal clinical outcomes.
Table 1. Comparison of Routine Tests That Assess Fetal Lung Maturity

<table>
<thead>
<tr>
<th>Method</th>
<th>TAT</th>
<th>Cost</th>
<th>Ease of Use</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence polarization</td>
<td>40 min</td>
<td>Moderate</td>
<td>Easy to perform. Requires sample to be filtered.</td>
<td>On demand, on-site.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fully automated.</td>
<td></td>
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<tr>
<td>Lamellar body counts</td>
<td>5-10 min</td>
<td>Inexpensive</td>
<td>Very easy to perform. Requires adequate mixing of specimen. Fully automated.</td>
<td>On demand, on-site.</td>
</tr>
<tr>
<td>L/S ratio</td>
<td>2-3 h</td>
<td>High</td>
<td>Technically demanding. Requires extraction techniques, followed by thin-layer chromatography. Results then visualized and quantified.</td>
<td>Requires dedicated personnel. Usually not on-site. Difficult to accommodate on-demand requests.</td>
</tr>
</tbody>
</table>

TAT indicates turnaround time from receipt of specimen; L/S, lecithin to sphingomyelin.

Materials and Methods

For this prospective study we used 100 fresh amniotic fluid specimens submitted to our laboratory (The Mary Birch Hospital for Women at Sharp Memorial Hospital, San Diego, a tertiary-care community-based hospital with 8,500 deliveries per year and a large high-risk obstetric population) for fetal lung maturity testing. All samples used in this study were collected by amniocentesis. Samples that contained visible blood, meconium, or mucous were not included in the study. Vaginal pool samples also were excluded owing to the large amount of mucous that may be present. (Mucous can artificially elevate lamellar body counts.) Lamellar body counts were performed in succession on each analyzer in accordance with each manufacturer’s instructions for platelet analysis. Both instruments use electrical impedance to count platelets. With this technology, particles are counted and measured as they pass through an aperture generating a pulse. The amplitude of each pulse is proportional to the volume of that particle. This method has been discussed in detail.4–6 Lamellar bodies have a particle size range of 1.7 to 7.3 fl (10-12) that allows them to be recognized and counted through the platelet apertures. Lamellar body reproducibility studies were performed on each analyzer and were measured by coefficients of variation (CVs), as well as by linearity studies using serially diluted amniotic fluid.

Amniotic fluid samples were run on the Abbott Cell Dyn 3500 using the open sample mode. This instrument automatically wipes the aspiration needle. A background count of zero was the acceptable limit for the platelet count to ensure that all blood had been purged from the system. The platelet aperture size is 60 μm, and pulses counted between 1 and 35 fl are included in the platelet data.11 The linearity for this platelet channel is 10 to 999 × 1012/L (10–999 × 109/L). Quality control was performed using the whole blood Cell Dyn 3000 Tri Level Reference Controls (Abbott Laboratories, Abbott Park, Ill) in accordance with manufacturer’s instructions.

Amniotic fluid samples run on the Coulter STKS were aspirated through the secondary mode (open sampler) of the analyzer with the differential mode disabled. Because this instrument does not automatically wipe the secondary sample needle, a 50% bleach solution was used to clean the needle, followed by rinsing with a balanced electrolyte solution (Isoton III, Coulter Diagnostics, Hialeah, Fla). A background count of zero was also the acceptable limit for the platelet count. The STKS range for platelet classification is 2 to 20 fl using a 50-mm aperture. The linearity of this platelet channel is 0 to 999 × 1012/L.12 Coulter Cell Controls (4C Plus, Coulter Diagnostics) were performed in accordance with the manufacturer’s instructions for platelet analysis.

The specimens were not centrifuged and were kept at room temperature until analysis. Samples were run within 8 hours of collection and were mixed on a mechanical rotator for 5 minutes or gently inverted several times.
The STKS requires 150 μL of specimen and the Cell Dyn requires 130 μL, using the open mode of each analyzer. After data collection, specimens associated with a delivery within 72 hours of amniocentesis were correlated with neonatal outcomes. The medical records of all infants admitted to the neonatal intensive care unit were reviewed, and the records of infants with any respiratory difficulty were reviewed by one neonatologist. The diagnosis of respiratory distress syndrome was based on the results of the clinical examination by the attending neonatologist, the chest roentgenogram, fetal lung maturity results (by a fluorescent polarization assay or by phospholipid thin-layer chromatography), and the response to artificial surfactant.

**Results**

The amniotic fluid used in this study had lamellar body counts ranging from 3 to 364 × 10^3/μL (3–364 × 10^9/L) on the Cell Dyn with a mean of 105 × 10^3/μL (105 × 10^9/L). The STKS counts ranged from 3 to 195 × 10^3/μL (3–195 × 10^9/L) and had a mean of 53 × 10^3/μL (53 × 10^9/L). The samples used in this study represented a broad range of pulmonary maturity, with gestational ages ranging from 29 to 39 weeks (Fig 2).

Lamellar body reproducibility studies on both instruments exhibited CVs of less than 8%. Linearity study results were very good, with average correlation coefficients of 0.99 on each analyzer. Based on these data, a single whole number was obtained from the platelet channel and used as a data point.

The scatter diagram for the comparison of the Cell Dyn 3500 and the STKS is shown in Figure 3. The results from the Cell Dyn 3500 exhibited strong correlation with the results from the STKS as demonstrated by a correlation coefficient of 0.99 and a slope of 0.467, yielding a regression equation of STKS = 3.91 + 0.467(Cell Dyn 3500).

Evaluation of the 100 cases revealed that 67 infants were delivered within 72 hours of specimen collection. Three of these infants had respiratory distress syndrome. The lamellar body counts for the neonates in whom the syndrome developed are listed in Table 2.
Comment
Amniotic fluid testing capabilities have progressed extensively during the last decade, with various laboratory test methods proposed and used to evaluate fetal lung maturity. The very number of tests reflects the dissatisfaction of clinicians and laboratory professionals with current testing, including the lecithin/sphingomyelin ratio, which is especially time consuming and labor intensive, resulting in a prolonged turnaround time. Fluorescence polarization assays are less labor-intensive and are automated but still incur a 40-minute turnaround time for results.

A rapid and reliable test for lung maturity allows the physician to expedite a specific management plan for the patient. Furthermore, by incorporating an algorithm of tests to predict lung maturity, laboratory personnel and testing methods are used most effectively. Lamellar body counts are inexpensive, rapid, and effective as the initial test for assisting in the prediction of fetal pulmonary maturity and can be performed using a small amount of amniotic fluid. We used freshly collected amniocentesis specimens; however, refrigerated (2°C–8°C) amniotic fluid specimens may be analyzed up to 72 hours after collection without a statistically significant change in value.13

The principal objective of our study was to compare the clinical performance of the Coulter STKS with the Abbott Cell Dyn 3500 whole blood analyzer. We found a good correlation between instruments; noting, however, that higher counts were consistently obtained with the Cell Dyn 3500. This difference is due in part to the broader platelet counting range and aperture size of the Cell Dyn 3500. Although a linear relationship existed between the analyzers, careful consideration must be given to the interpretation of instrument values when establishing lamellar body count ranges to be indicative of lung maturity. At our institution, we use the Cell Dyn 3500 for investigational purposes and are in the process of statistical analyses to determine the lamellar body count that indicates lung maturity.

Conclusion
Alteration of technique and the use of various analyzers can yield different ranges of lamellar body counts to indicate pulmonary maturity. Institutions will be faced with establishing and incorporating instrument-specific reporting protocols and reference ranges that include the analyzer and method used. Potential confusion may occur among physicians and laboratory professionals who are accustomed to the previously published lamellar body count ranges for fetal lung maturity using Coulter instrumentation.14 As with all testing methods, lamellar body counts should be used in conjunction with the patient’s clinical manifestations and other diagnostic procedures. Correlation with neonatal outcomes should precede acceptance of this method.©

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References