Validation of Lamellar Body Counts Using Three Hematology Analyzers

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Key Words: Fetal lung maturity; Fluorescent polarization; Lamellar body count

Abstract

The lamellar body count (LBC) represents an alternative method to the TDx-FLM II (Abbott Laboratories, Abbott Park, IL), which is planned to be discontinued, for assessing fetal lung maturity. Our objective was to validate the LBC on 3 hematology analyzers (Coulter LH 750 and Coulter Ac·T diff2, Beckman Coulter, Brea, CA; and Sysmex XE-2100, Sysmex, Mundelein, IL) to serve as a template for other laboratories attempting to perform in-house validation.

Intra-assay and interassay coefficients of variation ranged from 1.7% to 21.8% and 1.9% to 7.1%, respectively, and all analyzers demonstrated excellent linearity. Whole blood and meconium were shown to interfere with LBCs, and specimens with these contaminants should be tested using phosphatidyl glycerol. With a TDx-FLM II cutoff of 55 mg/g or more and an LBC cutoff of 50,000/μL or more for maturity, concordance between the TDx-FLM II and the LBC on all instruments was poor (<80% in all cases). Concordance between hematology analyzers was excellent (≥94%). When laboratories are performing in-house validations, they should not correlate LBC with TDx-FLM II results without outcome data. Correlation with another validated LBC method is preferred.

Respiratory distress syndrome (RDS) of the newborn is the seventh most common cause of newborn mortality in the United States. It is caused by a deficiency in the quantity and/or composition of pulmonary surfactant produced by the neonatal lung, which leads to alveolar collapse and neonatal hypoxia shortly after birth. The incidence of RDS decreases with increasing gestational age such that the risk of RDS is less than 5% at gestational ages of 37 weeks or more. Maternal steroid administration for the prevention of RDS and neonatal surfactant therapy for treatment has led to a significant decrease in RDS-associated mortality. Decisions regarding treatment and management rely partially on the results of fetal lung maturity (FLM) tests.

The lamellar body count (LBC) is a rapid and quantitative method for assessing FLM. Lamellar bodies are densely packed layers of phospholipid that represent a storage form of pulmonary surfactant. The similarity of lamellar body diameter (1-5 μm) to platelet diameter (2-4 μm) allows the rapid enumeration of lamellar bodies in amniotic fluid by using the platelet channel of an automated hematology analyzer. Numerous outcome studies have established the clinical usefulness of LBCs for predicting FLM.

The American College of Obstetricians and Gynecologists has advised documentation of FLM when an elective delivery is planned before 39 weeks of gestation. Owing to the strong predictive value of a mature result by any FLM test, a cascade or sequential testing approach for lung maturity has been recommended. The cascade algorithm uses a rapid test for an initial assessment of lung maturity and, if results are immature or transitional, requires additional testing until a mature result is obtained or all test options have been exhausted.
The most commonly used rapid FLM test is the TDx-FLM II assay (Abbott Laboratories, Abbott Park, IL) that determines the ratio of surfactant to albumin by fluorescence polarization.\textsuperscript{15,16} However, the instrument used to perform the TDx-FLM II assay is being retired by the manufacturer, which will require laboratories that currently perform the test to identify an alternative testing strategy.

Because the LBC is a laboratory-developed test, it must be thoroughly validated before clinical use. An important component of this validation is the establishment of cutoff values to be used for result interpretation of FLM. Several outcome studies have identified an LBC cutoff of 50,000/μL or more using Beckman Coulter instruments to identify lung maturity.\textsuperscript{6,7,11,17-21} While outcome-based studies are preferred for validating the clinical performance of FLM tests, the declining use of the FLM test and the low prevalence of RDS makes this virtually impossible, even for very large medical centers.\textsuperscript{22} Thus, the objectives of this study were to analytically validate 3 hematology analyzers—the Coulter LH 750 and Coulter Ac·T diff2, Beckman Coulter, Brea, CA; and the Sysmex XE-2100, Sysmex, Mundelein, IL—for determining the LBC and to compare results from these instruments with those of the TDx-FLM II. This study can also serve as a template for others wanting to perform an analytic validation of the LBC in their laboratory.

Materials and Methods

Study Samples

Residual amniotic fluid samples submitted for physician-ordered FLM testing were used. Institutional review board approval was obtained for this study. Samples were used in this study if sufficient volume remained for LBC testing after all clinical tests were completed. Samples were stored at 4°C for up to 12 days before performing the LBC with the exception of samples used for stability studies, which were stored as indicated.

Analytic Methods

TDx-FLM II analysis was performed at Barnes-Jewish Hospital, St. Louis, MO, on an Abbott TDx-FLM II analyzer according to the manufacturer’s instructions. The upper and lower limits of detection for the TDx-FLM II assay were 160 and 10 mg/g, respectively. The cutoff for indicating FLM was 55 mg/g or more, as recommended by the manufacturer.

The LBC was performed using the platelet channel of a Coulter LH 750 at Barnes-Jewish Hospital, St. Louis, MO; a Coulter Ac·T diff2 at ARUP Laboratories, Salt Lake City, UT; and a Sysmex XE-2100 at St Louis Children’s Hospital, St Louis. The cutoff for indicating FLM was 50,000/μL or more based on outcome studies using Beckman Coulter hematology analyzers.\textsuperscript{6,7,11,17-21}

Samples for LBC testing were processed without centrifugation, as described in a consensus protocol.\textsuperscript{21} Briefly, amniotic fluid samples were mixed by inversion for a minimum of 2 minutes to ensure resuspension of lamellar bodies, and the lamellar bodies were then enumerated using the CBC mode of the analyzer. The results from the platelet channel were recorded as the LBC.

Stability and Effect of Sample Freezing

To assess the stability of lamellar bodies, 4 samples with immature, transitional, or mature TDx-FLM II results were stored at 4°C for up to 33 days (range, 20-33 days) and tested on at least 6 separate occasions (range, 6-9). Aliquots of 25 samples were stored at –80°C for at least 24 hours (range, 16-156 days; median, 74 days) to ascertain the effect of a single freeze-thaw cycle.

For electron microscopy studies, 2 aliquots of 3 amniotic fluid samples were prepared. One aliquot was stored at 4°C and the other at –20°C for 24 hours. The –20°C aliquot was thawed at 37°C, and both samples were then centrifuged for 15 minutes at 1,000g. The pellets were sequentially fixed in 2% paraformaldehyde/2.5% glutaraldehyde and 1% osmium tetroxide. Pellets were dehydrated, embedded in EMBed 812 resin (Electron Microscopy Sciences, Hatfield, PA), sectioned, and stained with 1% uranyl acetate and lead citrate. Sections were viewed on a Hitachi H7500 transmission electron microscope (Hitachi High Technologies America, Pleasanton, CA). The 4°C and the –20°C samples were prepared and viewed simultaneously. The diameter of at least 530 lamellar bodies was measured manually by comparing the scale bar with each lamellar body for each condition tested.

Interference From Whole Blood and Meconium

To assess the potential interference from whole blood contamination, freshly collected EDTA anticoagulated whole blood was added to pooled amniotic fluid samples. Ten pools of amniotic fluid were prepared that spanned an LBC of 2,000 to 30,000/μL. Meconium solutions with a final concentration of 55 mg/g or more based on outcome studies using Beckman Coulter hematology analyzers.\textsuperscript{6,7,11,17-21} Meconium was added to pooled amniotic fluid samples. Ten pools for each condition tested.

For each pool, whole blood was added to pooled amniotic fluid samples. Ten pools for each condition tested. A parallel series of dilutions was prepared using 0.9% saline. Specimens were incubated at room temperature on a rocker for 30 minutes before determining the LBC using the Coulter Ac·T diff2.

To assess the potential interference from meconium contamination, meconium was added to pooled amniotic fluid samples. Eight pools of amniotic fluid were prepared that spanned an LBC of 2,000 to 30,000/μL. Meconium specimens were obtained from each of 3 newborns and added to 0.9% saline to achieve 3 meconium solutions with a final concentration of 55 mg/g or more based on outcome studies using Beckman Coulter hematology analyzers.\textsuperscript{6,7,11,17-21}
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concentration of 10 g/L. For each amniotic fluid pool, a meconium solution was added to achieve a meconium concentration range of 0.02 to 5.0 g/L. To control for the dilution effect, a parallel series of dilutions was prepared using 0.9% saline. Specimens were incubated on a room temperature rocker for 30 minutes before determining the LBC using the Coulter Ac·T diff2.

Statistics

Linearity, stability, interference, and method comparison data were evaluated with GraphPad Prism software, version 4.0 (GraphPad, San Diego, CA). GraphPad Prism, version 5.0, was used for Deming regression analysis.

Results

Imprecision

Intra-assay and interassay imprecision studies were performed with amniotic fluid samples. Intra-assay imprecision was evaluated by determining the LBC in 3 amniotic fluid samples 11 times. For the Coulter LH 750, intra-assay imprecision was as follows: 1.8%, mean 25,300 counts/μL; 2.5%, mean 33,100 counts/μL; and 2.1%, mean 53,600 counts/μL. The Sysmex XE-2100 had the following intra-assay imprecision: 4.7%, mean 24,500 counts/μL; 4.3%, mean 35,000 counts/μL; and 2.3%, mean 54,600 counts/μL. The Coulter Ac·T diff2 had the following intra-assay imprecision: 21.8%, mean 14,600 counts/μL; 12.2%, mean 27,500 counts/μL; and 5.8%, mean 59,800 counts/μL. Interassay imprecision was assessed by measuring the LBC in 4 amniotic fluid samples on 6 separate days. For the Coulter LH 750, interassay imprecision was as follows: 5.1%, mean 14,800 counts/μL; 4.8%, mean 28,700 counts/μL; 3.4%, mean 54,700 counts/μL; and 1.9%, mean 66,000 counts/μL. Interassay imprecision for the Sysmex XE-2100 was as follows: 7.1%, mean 13,800 counts/μL; 3.4%, mean 30,300 counts/μL; 1.3%, mean 58,200 counts/μL; and 2.9%, mean 67,300 counts/μL.

Linearity

Linearity was determined by using mixtures of amniotic fluid specimens with a high and a low LBC to produce final proportions (high/low) of 1.0, 0.75, 0.50, 0.25, 0.10, and 0.05, tested in triplicate, and the results were compared against the expected LBC. The maximum deviation from the target recovery was 14.3% at a concentration of 14,000 counts/μL on the Coulter LH 750 analyzer. Comparison between the measured and expected results yielded the following linear regression equations: Coulter LH 750, $y = 1.027x - 0.821$; $r^2 = 0.997$. Sysmex XE-2100, $y = 0.981x - 0.446$; $r^2 = 0.999$. Coulter Ac·T diff2, $y = 1.061x - 1.628$; $r^2 = 0.996$. These equations and coefficients of determination indicate excellent linearity of the hematology analyzers in the concentration range from 1,000 to 80,000 counts/μL.
Stability and Effect of Sample Freezing

The stability of lamellar bodies in amniotic fluid at 4°C is shown in Figure 2. The Coulter Ac·T diff2 analyzer had the largest deviation from the initial LBC value at a lamellar body concentration of 20,000 counts/μL with a coefficient of variation of 15%.

The effect of a single freeze-thaw cycle on the LBC is shown in Figure 3. In all 25 samples (100%), the LBC decreased after a single freeze-thaw cycle. The mean (SD) percentage LBC decreases on the Coulter LH 750, Sysmex XE-2100, and Coulter Ac·T diff2 were 29% (9.7%), 38% (10.7%), and 25% (13.5%), respectively. The mean (SD) percentage LBC decrease for samples across all instruments was 31% (12.6%) with a range of 3% to 57%. The percentage decrease in the LBC did not correlate with the initial lamellar body concentration.

Electron microscopy of samples before and after freezing at –20°C for 24 hours revealed that lamellar bodies from thawed samples are qualitatively different from those that have never been frozen. A single freeze-thaw cycle resulted in a nearly complete absence of densely packed lamellar bodies Image 1. Freezing also caused an increase in the number of lamellar bodies with a diameter of less than 1 μm Table 1.

Interference From Whole Blood and Meconium

The effect of whole blood and meconium on the LBC is shown in Figure 4. In the 10 amniotic fluid pools to which whole blood was added, the LBC decreased in a dose-dependent manner with increasing amounts of RBCs. The mean (SD) percentage LBC decreases were 1.5% (5.4%) for RBC counts of 31,000 × 10⁶/L (0.031 × 10¹²/L) or less and 26.3% (16.8%) for RBC counts of more than 31,000 × 10⁶/L (0.031 × 10¹²/L).
Small blood clots were observed in 10, 8, and 2 of 10 pools at mean RBC counts of $490,000 \times 10^6/L$ (0.49 $\times 10^{12}$/L), $110,000 \times 10^6/L$ (0.11 $\times 10^{12}$/L), and $50,000 \times 10^6/L$ (0.05 $\times 10^{12}$/L), respectively.

The addition of meconium to 8 amniotic fluid pools caused the LBC to increase dramatically from the baseline values. The mean (SD) percentage LBC increases were 9.4% (27.9%) at a meconium concentration of 0.5 g/L or less and 117.5% (235.2%) at a concentration of more than 0.5 g/L.

**Method Comparison**

Results from the TDx-FLM II, Coulter LH 750, Sysmex XE-2100, and Coulter Ac·T diff2 methods were compared using Deming regression, as shown in **Figure 5**.

Comparisons between the TDx-FLM II result and the LBC yielded the following regression equations: Coulter LH 750, $y = 1.04x - 17.20$; $r = 0.75$; $x = 22.93$; Sysmex XE-2100, $y = 1.04x - 17.20$; $r = 0.75$; $x = 22.93$.

**Table 1**

Particle Size by Electron Microscopy of Lamellar Body Counts Before and After Freezing

<table>
<thead>
<tr>
<th>Amniotic Fluid Storage Temperature</th>
<th>Particle Size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4°C (n = 530)</td>
</tr>
<tr>
<td>&lt;1</td>
<td>338 (63.8)</td>
</tr>
<tr>
<td>1-4</td>
<td>185 (34.9)</td>
</tr>
<tr>
<td>&gt;4</td>
<td>7 (1.3)</td>
</tr>
</tbody>
</table>

*Particle size was measured manually by measuring particles on electron microscopy scans; n is the number. Data are given as number (percentage) of particles counted.

**Image 1** Transmission electron microscopy photomicrographs of lamellar bodies. Amniotic fluid samples that underwent a single freeze-thaw cycle at $-20^\circ C$ showed a decrease in densely packed lamellar bodies. **A** and **B**, Prefreeze lamellar bodies ($>40,000$). **C** and **D**, Postfreeze lamellar bodies ($>40,000$).
We also sought to establish the relationships between the Coulter LH 750 and 2 other hematology analyzers through Deming regression analysis, which demonstrated very good correlation with coefficients of 0.95 or more in all cases. The regression equations are as follows:

- Coulter LH 750, \( y = 1.06x - 0.99; \) \( r = 0.99; \) \( S_y, x = 5.72; \) and Coulter Ac-T diff2, \( y = 1.02x - 1.75; \)

For the Coulter LH 750 and Sysmex XE-2100, the equations are:

- Sysmex XE-2100, \( y = 1.06x - 0.99; \) \( r = 0.99; \) \( S_y, x = 5.72; \) and Coulter Ac-T diff2, \( y = 1.02x - 1.75; \)

The correlations for the Coulter LH 750 and TDx-FLM II are:

- \( y = 1.19x - 23.41; \) \( r = 0.74; \) \( S_y, x = 25.54; \) Coulter AcTdiff2,
- \( y = 0.901x - 19.45; \) \( r = 0.75; \) \( S_y, x = 21.99. \)

There was poor correlation between the TDx-FLM II and all LBC methods, as indicated by nonparametric correlation coefficients of 0.75 or less. Overall concordance between TDx-FLM II and the Coulter LH 750 was 76.2% (80/105) and between TDx-FLM II and the Sysmex XE-2100 was 77.1% (81/105) and TDx-FLM II and Coulter Ac·T diff2 was 70% (31/44).

The tables and figures show the comparison of results from the TDx-FLM II and the lamellar body count (LBC). Concordance and Deming regression analysis between the TDx-FLM II and the Coulter LH 750, Sysmex XE-2100, and Coulter Ac·T diff2 are presented. Cutoffs for maturity are indicated by the dotted lines (55 mg/g for TDx-FLM II and 50,000/μL for the LBC).
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decrease because we measured only an 11% increase in the number of lamellar bodies smaller than 1 μm, which does not entirely explain the mean 31% decrease after freezing.

One limitation of the LBC test is the lack of commercially available quality control material. The demonstrated stability of lamellar bodies for more than 1 month at 4°C is an important practical consideration for laboratories establishing internal quality controls that may need to use pooled patient amniotic fluid specimens and for laboratories to share samples for assay validation.

The addition of whole blood to amniotic fluid has previously been reported to cause a biphasic interference with the LBC by initially increasing the result followed by a subsequent decrease during a 10- to 60-minute incubation as lamellar bodies are entrapped in fibrin aggregates.23,24 This study demonstrated that the LBC began to show a decrease as the amount of whole blood increased (Figure 4A). At a mean RBC count of 50,000 × 10⁶/L (0.05 × 10¹²/L), small blood clots were clearly visible in 20% of samples, supporting the hypothesis that lamellar bodies are trapped in a fibrin matrix. There was no observed effect of blood contamination when the RBC count was 31,000 × 10⁶/L (0.031 × 10¹²/L) or less. It is interesting that this is the same threshold of blood contamination that was identified as being acceptable for the TDx-FLM II assay.25

The addition of meconium to amniotic fluid samples dramatically increased the LBC (Figure 4B), likely owing to the presence of particles in the meconium that are detected by the platelet channel of the cell counter. Thus, specimens that are contaminated with meconium should not be expected to produce accurate results.

Lamellar bodies should not be counted in amniotic fluid contaminated with blood (RBC counts >31,000 × 10⁶/L [0.031 × 10¹²/L]) or meconium. Specimens with this type of contamination before

Discussion

The LBC is a simple, rapid, reliable, and inexpensive FLM test performed using the platelet channel of automated hematology analyzers. In the light of the impending discontinuation of the rapid and highly used TDx-FLM II assay, the LBC is a logical replacement test for rapid FLM testing. However, it is important to thoroughly validate this laboratory-developed test.

We determined the analytic characteristics of the Coulter LH 750, Sysmex XE-2100, and Coulter Ac·T diff2 hematology analyzers for determining the LBC in amniotic fluid. These methods are linear throughout the analytic range from 1,000 to 80,000 counts/μL, with imprecision ranging from 1.8% to 21.8%.

The stability of lamellar bodies was examined when stored refrigerated at 4°C or frozen at –80°C. Lamellar bodies were stable for at least 33 days when stored at 4°C with an imprecision ranging from 1.2% to 15.1%. A single freeze-thaw cycle caused a 31% mean decrease in the LBC. When amniotic fluid was examined by electron microscopy, it was clear that freezing resulted in the disappearance of nearly all densely packed lamellar bodies and an increase in the number of very small (<1 μm) particles. We hypothesize that at least part of the decrease in the LBC is due to a shift in particle size, and the smallest particles, therefore, are not counted in the 2- to 20-fl platelet window. This may not account for the entire decrease because we measured only an 11% increase in the number of lamellar bodies smaller than 1 μm, which does not entirely explain the mean 31% decrease after freezing.

One limitation of the LBC test is the lack of commercially available quality control material. The demonstrated stability of lamellar bodies for more than 1 month at 4°C is an important practical consideration for laboratories establishing internal quality controls that may need to use pooled patient amniotic fluid specimens and for laboratories to share samples for assay validation.

The addition of whole blood to amniotic fluid has previously been reported to cause a biphasic interference with the LBC by initially increasing the result followed by a subsequent decrease during a 10- to 60-minute incubation as lamellar bodies are entrapped in fibrin aggregates.23,24 This study demonstrated that the LBC began to show a decrease as the amount of whole blood increased (Figure 4A). At a mean RBC count of 50,000 × 10⁶/L (0.05 × 10¹²/L), small blood clots were clearly visible in 20% of samples, supporting the hypothesis that lamellar bodies are trapped in a fibrin matrix. There was no observed effect of blood contamination when the RBC count was 31,000 × 10⁶/L (0.031 × 10¹²/L) or less. It is interesting that this is the same threshold of blood contamination that was identified as being acceptable for the TDx-FLM II assay.25

The addition of meconium to amniotic fluid samples dramatically increased the LBC (Figure 4B), likely owing to the presence of particles in the meconium that are detected by the platelet channel of the cell counter. Thus, specimens that are contaminated with meconium should not be expected to produce accurate results.

Lamellar bodies should not be counted in amniotic fluid contaminated with blood (RBC counts >31,000 × 10⁶/L [0.031 × 10¹²/L]) or meconium. Specimens with this type of contamination before
contamination can be evaluated with a phosphatidyl glycerol test (as long as phosphatidyl glycerol is not expressed as a ratio to sphingomyelin).

Overall, results from the TDx-FLM II assay demonstrated poor correlation ($r \leq 0.75$) and concordance ($<80\%$) with the LBC result obtained from all 3 hematology analyzers. This was most evident in samples with a TDx-FLM II result of 55 mg/g or more (ie, a mature result). The most common discrepant result was for the TDx-FLM II to predict FLM, whereas the LBC did not. Such a discrepancy is not unexpected considering that the 2 assays use different methods. The TDx-FLM II is a biochemical assessment of the ratio of pulmonary surfactant to albumin, whereas the LBC is a biophysical measurement of the absolute number of lamellar bodies. It is important to note that the TDx-FLM II and LBC results were clinically concordant when the TDx-FLM II result was less than 55 mg/g.

Despite an overall concordance of only 79%, a recent outcome-based study by Haymond et al demonstrated a predictive value of 99% for a mature result by the TDx-FLM II or LBC. Numerous clinical outcome studies have also reported similarly high mature predictive values for the LBC. These outcome-based reports provide evidence that the LBC can be used to reliably predict FLM. Therefore, we suggest that laboratory professionals performing an LBC method validation do not put too much emphasis on TDx-FLM II and LBC comparison studies in the absence of outcome data. Comparison studies with a previously validated LBC method are preferred.

Method comparisons of the Coulter LH 750, the Sysmex XE-2100, and Coulter Acc-T diff2 hematology analyzers demonstrated excellent correlation ($r > 0.95$ in all cases) and were analytically highly concordant for all results ($\geq 94\%$ in all cases). Previous studies have highlighted the need for analyzer-specific LBC clinical decision limits, particularly with cell counters that use different platelet counting methods. Because all 3 of the hematology analyzers in this study use conventional impedance principles to count platelets, it is not surprising that these instruments show excellent agreement (Figure 6). The data presented herein indicate that the published consensus LBC cutoff of 50,000 counts/μL or more for lung maturity, which was primarily derived from outcome-based studies with Coulter-brand hematology analyzers, is also appropriate for the Sysmex XE-2100. Although 2 outcome-based studies have suggested lower maturity cutoffs on Sysmex instruments of 30,000 counts/μL, both studies used centrifuged amniotic fluid samples, which is known to decrease the LBC and is not recommended in the consensus protocol. An outcome-based study that did not centrifuge samples suggested a lamellar body maturity cutoff of 45,000 counts/μL on the Sysmex XE-800, which is similar to our proposed cutoff of 50,000 counts/μL on the Sysmex XE-2100.

The widespread availability of hematology analyzers underscores the potential for many laboratories to offer the LBC as a rapid and inexpensive assessment of FLM. In this report, we have outlined the necessary studies for developing and validating the LBC test for routine clinical use. While commercial lamellar body quality control materials are not yet available, our study demonstrated LBC stability in amniotic fluid for at least 1 month if specimens are refrigerated. If a laboratory wants to offer the LBC as a replacement for the widely used TDx-FLM II assay, it is important that it initiate validation studies and educate clinicians about the method, specimen requirements, rapid turnaround time, and clinical usefulness that this test provides for the assessment of FLM.

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**References**


