This document provides guidelines for the use of automated cell counting to enumerate lamellar bodies in amniotic fluid. It describes the different counting technologies used in automated cell counters as well as methods laboratorians can use to verify/validate the lamellar body count test.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.
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Assessment of Fetal Lung Maturity by the Lamellar Body Count; Approved Guideline

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Abstract

Clinical and Laboratory Standards Institute document C58-A—Assessment of Fetal Lung Maturity by the Lamellar Body Count; Approved Guideline provides guidance to laboratory professionals and manufacturers involved in the development of devices and materials related to the enumeration of lamellar bodies in amniotic fluid as a test of fetal lung maturity (FLM). Physicians use FLM tests to weigh the potential risks to a newborn of developing respiratory distress syndrome caused by a deficiency of pulmonary surfactant. Pulmonary surfactant decreases the surface tension of the hydrated inner layer of alveoli and prevents their collapse during exhalation. Pulmonary surfactant is packaged into lamellar bodies that are secreted from pneumocytes. The enumeration of lamellar bodies in amniotic fluid can be used as a test of FLM. This document provides guidelines for the use of automated cell counting to perform the lamellar body count test and describes methods to assist in test verification and validation.


The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org. If your organization is not a member and would like to become one, and to request a copy of the catalog, contact us at: Telephone: 610.688.0100; Fax: 610.688.0700; E-Mail: customerservice@clsi.org; Website: www.clsi.org.
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Foreword

Development of the fetal lung can be divided into four stages: the pseudoglandular, canalicular, saccular, and alveolar stages. The first stage results in the development of three lung lobes on the right side and two on the left side. The second stage is remarkable for the differentiation of type I and type II pneumocytes and the first appearance of surfactant. The third stage involves formation of clusters of wide spaces in the peripheral airways. Finally, the fourth stage involves the formation of alveoli. It is during this stage that type II pneumocytes increase production of pulmonary surfactant. Lung development continues for approximately eight years.

Pulmonary surfactant functions to coat the alveolar epithelium and decrease the surface tension of the hydrated inner layer of alveoli. When surface tension is high and the alveolar radius is small, very high air pressure is needed to prevent alveolar collapse. Surfactant decreases the air pressure required to keep the alveoli from collapsing. Surfactant is composed of approximately 90% phospholipid and 10% protein, and is packaged into layered storage granules called lamellar bodies that begin to synthesize around 24 weeks of gestation. Lamellar bodies are secreted by the type II pneumocyte and unfold to form tubular myelin and other large aggregates that are adsorbed onto the hydrated inner layer of the alveoli.

Respiratory distress syndrome (RDS) in premature infants is caused by developmental insufficiency of pulmonary surfactant production and structural immaturity of the lungs. Clinically, RDS presents with hypoxia, hypercapnia, and acidosis. Preventing premature birth is the most effective way to prevent RDS. Alternatively, administration of steroids to the mother can be used to accelerate lung surfactant production. Treatment of preterm newborns after birth with exogenous surfactant can be effective in preventing and treating RDS.

Fetal lung maturity (FLM) tests are used by physicians to weigh the risk of developing RDS if the newborn is delivered against the risk to the mother by continuing the gestation. To be clinically useful, FLM tests should possess high diagnostic sensitivity for RDS and a high predictive value of a mature result. Interestingly, no studies have addressed the impact of FLM testing on improving patient care. Studies have indicated that the frequency of physician-ordered FLM testing is decreasing. This likely reflects a decrease in elective deliveries in response to studies that demonstrate more adverse outcomes in infants delivered before 39 weeks of gestation. However, despite the decreased use of FLM tests, physicians still report that they rely on them for clinical decision making.

Key Words

Amniotic fluid, fetal lung maturity, lamellar bodies, lamellar body count, respiratory distress syndrome
Assessment of Fetal Lung Maturity by the Lamellar Body Count; Approved Guideline

1 Scope

This document provides guidelines for the use of automated cell counting to enumerate lamellar bodies in amniotic fluid. It describes the different counting technologies used in automated cell counters as well as methods laboratorians can use to verify/validate the lamellar body count (LBC) test.

The intended users of this guideline are laboratory directors, medical technologists, laboratory supervisors, and pathologists, as well as in vitro diagnostic manufacturers involved in the development of devices and materials related to LBC testing.

This guideline does not provide guidance on how to establish the clinical utility of the LBC for fetal lung maturity (FLM).

2 Introduction

In 1988, Stuart Dubin used light scattering to study the refractive index of amniotic fluid as a measure of FLM.6,7 His observations led to the determination of the lamellar body number density (lamellar bodies per unit volume; typically between 10 000 and 200 000/µL) and demonstrated that lamellar bodies are similar in size to platelets (1.7–7.3 fl or 1–5 µm vs 5–7 fl or 2–4 µm, respectively). The latter finding suggested that lamellar bodies could be quantified using the platelet channel of an automated cell counter.

Since those early observations, lamellar body counting has proven to have many advantages over other tests of FLM, including:

- Rapid turnaround time
- Low reagent cost
- Wide availability
- Low degree of technical difficulty
- Low volume of amniotic fluid required
- Excellent clinical performance

3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to “standard precautions.” Standard precautions are guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the Centers for Disease Control and Prevention.8 For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.9
4 Terminology

4.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI’s consensus process for development and revision of standards and guidelines focuses on harmonization of terms to facilitate the global application of standards and guidelines.

4.2 Definitions

accuracy (measurement) – closeness of agreement between a measured quantity value and a true quantity value of a measurand (JCGM 200:2008).10

analyte – component represented in the name of a measurable quantity (ISO 17511)11; NOTE 1: In the type of quantity “mass of protein in 24-hour urine,” “protein” is the analyte. In “amount of substance of glucose in plasma,” “glucose” is the analyte. In both cases, the long phrase represents the measurand (ISO 17511)11; NOTE 2: In the type of quantity “catalytic concentration of lactate dehydrogenase isoenzyme 1 in plasma,” “lactate dehydrogenase isoenzyme 1” is the analyte (ISO 18153).12

amniotic fluid – the fluid surrounding a fetus within the amnion.

bias – the difference between the expectation of the test results and an accepted reference value (ISO 3534-1).13

calibration – operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication (JCGM 200:2008)10; NOTE: According to the US Code of Federal Regulations, calibration is a process of testing and adjusting an instrument or test system to establish a correlation between the measurement response and the concentration or amount of the substance that is being measured by the test procedure (42 CFR § 493.2).14

diagnostic sensitivity – the proportion of patients with a well-defined clinical disorder whose test values are positive or, as in the case with the lamellar body count, below a defined decision limit (ie, a positive result and identification of the patients who have a disease); NOTE 1: The clinical disorder must be defined by criteria independent of the test under consideration; NOTE 2: The term “diagnostic sensitivity” (Europe) is equivalent to “clinical sensitivity” (United States).

error (measurement)//measurement error – measured quantity value minus a reference quantity value (JCGM 200:2008).10

fetal lung immaturity – the absence of lung maturity in a fetus, primarily due to an insufficient quantity of pulmonary surfactant that is nearly always associated with preterm birth.

fetal lung maturity (FLM) – the presence of a functional fetal lung as indicated by an adequate amount of pulmonary surfactant.
The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The QSEs are as follows:

- Organization
- Personnel
- Process Management
- Nonconforming Event Management
- Customer Focus
- Purchasing and Inventory
- Documents and Records
- Assessments
- Facilities and Safety
- Equipment
- Information Management
- Continual Improvement
- Personnel
- Process Management
- Nonconforming Event Management
- Customer Focus
- Purchasing and Inventory
- Documents and Records
- Assessments
- Facilities and Safety
- Equipment
- Information Management
- Continual Improvement
- Process Management
- Documents and Records
- Assessments
- Information Management
- Continual Improvement
- Continual Improvement

C58-A addresses the QSE indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

C58-A addresses the clinical laboratory path of workflow processes indicated by an “X.”
Related CLSI Reference Materials*


EP05-A2  Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (2004). This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturers’ precision performance claims and determining when such comparisons are valid; as well as manufacturers’ guidelines for establishing claims.


EP15-A2  User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition (2006). This document describes the demonstration of method precision and trueness for clinical laboratory quantitative methods utilizing a protocol designed to be completed within five working days or less.

EP17-A  Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (2004). This document provides guidance for determining the lower limit of detection of clinical laboratory methods, for verifying claimed limits, and for the proper use and interpretation of the limits. An NCCLS-IFCC joint project.

GP29-A2  Assessment of Laboratory Tests When Proficiency Testing Is Not Available; Approved Guideline—Second Edition (2008). This document offers methods to assess test performance when proficiency testing (PT) is not available; these methods include examples with statistical analyses. This document is intended for use by laboratory managers and testing personnel in traditional clinical laboratories as well as in point-of-care and bedside testing environments.

M29-A3  Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005). Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.
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