CLINICAL AND LABORATORY STANDARDS INSTITUTE®

November 2011

C58-A

Assessment of Fetal Lung Maturity by the Lamellar Body Count; Approved Guideline

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.

Clinical and Laboratory Standards Institute

Setting the standard for quality in medical laboratory testing around the world.

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advances in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential and may be submitted by anyone, at any time, on any document. All comments are managed according to the consensus process by a committee of experts.

Appeal Process

When it is believed that an objection has not been adequately considered and responded to, the process for appeal, documented in the CLSI *Standards Development Policies and Processes*, is followed.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

For additional information on committee participation or to submit comments, contact CLSI.

Clinical and Laboratory Standards Institute
P: +1.610.688.0100
F: +1.610.688.0700
www.clsi.org
standard@clsi.org

ISBN 1-56238-771-5 (Print)
ISBN 1-56238-772-3 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic)

C58-A
Vol. 31 No. 20

Assessment of Fetal Lung Maturity by the Lamellar Body Count; Approved Guideline

Volume 31 Number 20

David G. Grenache, PhD, DABCC, FACB
Agim Beshiri, MD
Ann M. Gronowski, PhD
Andra Kyle, RT, BSc
Timothy G. McManamon, PhD, DABCC
Melissa Singer, MT(ASCP)
Elizabeth Wiet, MT(ASCP)

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.

Clinical and Laboratory Standards Institute (CLSI). *Assessment of Fetal Lung Maturity by the Lamellar Body Count; Approved Guideline*. CLSI document C58-A (ISBN 1-56238-771-5 [Print]; ISBN 1-56238-772-3 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2011.

Suggested Citation

CLSI. *Assessment of Fetal Lung Maturity by the Lamellar Body Count; Approved Guideline.* CLSI document C58-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

Reaffirmed:
September 2016

Archived:
July 2020

ISBN 1-56238-771-5 (Print)
ISBN 1-56238-772-3 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic)

Contents

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.[1] 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.[2,3] 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.[4,5] However, despite the decreased use of FLM tests, physicians still report that they rely on them for clinical decision making.[3]

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

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.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
		M29				X C24 EP05 EP06 EP09 EP15 EP17 GP29				GP29	

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."

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
	X		X	X		X	X	

Related CLSI Reference Materials*

C24-A3 **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition (2006).** This guideline provides definitions of analytical intervals, planning of quality control procedures, and guidance for quality control applications.

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.

EP06-A **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (2003).** This document provides guidance for characterizing the linearity of a method during a method evaluation; for checking linearity as part of routine quality assurance; and for determining and stating a manufacturer's claim for linear range.

EP09-A2-IR **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (Interim Revision) (2010).** This document addresses procedures for determining the bias between two clinical methods, and the design of a method comparison experiment using split patient samples and data analysis.

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.

CLINICAL AND
LABORATORY
STANDARDS
INSTITUTE®

950 West Valley Road, Suite 2500, Wayne, PA 19087 USA

P: +1.610.688.0100 **Toll Free (US):** 877.447.1888 **F:** +1.610.688.0700

E: customerservice@clsi.org **www.clsi.org**

PRINT ISBN 1-56238-771-5

ELECTRONIC ISBN 1-56238-772-3