CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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C48-A

Application of Biochemical Markers of Bone Turnover in the Assessment and Monitoring of Bone Diseases; Approved Guideline

This guideline provides information on how biochemical markers of bone turnover can be applied to facilitate and harmonize data interpretation and to help answer clinical questions in the area of bone diseases.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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NOTE: This document is no longer being reviewed as part of the CLSI consensus process. However, because of its usefulness to segments of the health care community, it is available for its informational content.

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Application of Biochemical Markers of Bone Turnover in the Assessment and Monitoring of Bone Diseases; Approved Guideline

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Abstract

Bone markers are useful tools for the management of bone diseases, because they provide information that is different but complementary to bone mineral density (BMD) measurement. Problems in the measurement and interpretation of bone marker values are still hampering the optimal utility of this clinical tool. Most of these difficulties originate from problems related to the handling and control of analytical and biological variability of bone marker measurements. These sources of variability can be substantial and need to be controlled. In this context, certain applications of bone marker testing may have different requirements for the handling and control of these sources of variability. Finally, certain bone markers or bone marker tests may require special considerations, such as analyte stability. This document addresses these issues and provides information related to applications of bone marker measurements.

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Foreword

Bone, as a structural tissue, is subject to stress damage that can eventually lead to fracture if allowed to progress. However, bone has the ability to repair itself by a process called "bone remodeling" which consists of removal of older bone tissue (resorption) and replacement with new bone tissue (formation). Resorption and formation are interdependent, and the processes always begin with resorption of bone on the skeletal surface forming a resorption cavity. The cells responsible for resorption are osteoclasts. Bone formation begins when the resorption cavity is complete with deposition of bone matrix, 90% of which is Type I collagen followed by mineralization of this matrix with hydroxyapatite (see Figure 1). Through the first ten years of human growth, bone remodeling is accelerated and formation exceeds remodeling. Both processes are in balance during the second to approximately the fourth decade of life. Then, beginning about the fifth decade of life, resorption slightly exceeds formation, resulting in a net negative balance. Consequently, anything that accelerates the rate of remodeling also accelerates bone loss. Menopause is the most prevalent cause of accelerated bone loss.

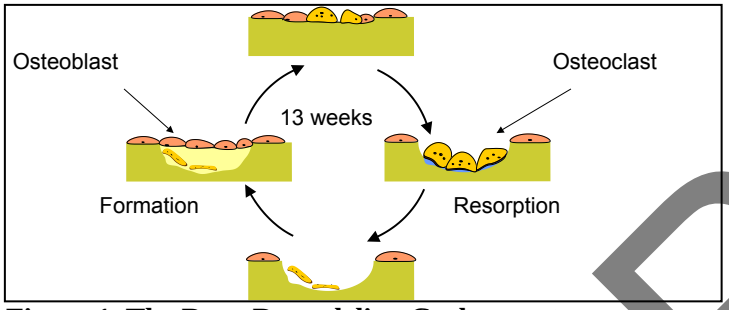

Figure 1. The Bone Remodeling Cycle

Bone status can be described by measuring bone mineral density, which provides information on both bone mineral content and bone fragility. Bone mineral density measurement, however, does not provide data on the rate of bone remodeling (whether formation exceeds or lags resorption). This information is obtained, qualitatively, by measuring biochemical bone markers (the biochemical substance produced or released during bone turnover). Both measurements complement each other and are needed to get a clear understanding of bone status. For example, while bone mineral density provides information about current bone status such as normal bone, osteopenic bone or osteoporotic bone, high bone marker values over an extended period of time indicate that the determined bone mineral density will decrease.

Several approaches listed in Table 1 have been developed to evaluate bone status in patients with metabolic bone diseases.

Table 1. Useful Methods in the Evaluation of Common Bone Diseases

Disease	Bone Mineral Density	Radiograph (X-Ray)	Radionuclide Scan	Biochemical Bone Markers
Osteoporosis	+	+*	–	+
Paget's Disease	–	+	+	+
Primary Hyperparathyroidism	+	+*	–	+
Osteomalacia	+	+	+	+
Metastatic Bone Disease	–	+	+	+

* if fractures are suspected
\+ method normally used for evaluation of disease
– method normally not used for evaluation of disease

While three of the methodologies mentioned in Table 1 provide information primarily about bone macrostructure, integrity, and quantity, only bone marker data provides information about bone turnover. BMD measurement provides data on the cumulative benefits and liabilities to the skeleton over the years; whereas bone markers provide information on the current metabolism of the whole skeleton.

Bone marker measurements have the advantage that changes in bone marker concentrations can also be seen earlier than changes in bone mineral density. For example, one study identified a response to antiresorptive treatment using bone mineral density measurement after one year (indicated by a 3% increase from baseline) and using osteocalcin as bone markers after three months (indicated by a -20% change from baseline).[1] Furthermore, changes in BMD measurements with osteoporosis do not always fully predict the observed effect on fracture risk.[2]

Bone markers can be measured in blood and urine and fall into two categories: (a) enzymes or other proteins secreted by osteoblasts or osteoclasts; and (b) substances produced during the formation or breakdown of Type I collagen, the primary protein forming the organic matrix in bone.[2]

The markers of *bone resorption* are degradation products of bone. Both bone mineral (primarily calcium) and bone matrix (primarily Type I collagen) are broken down and released into the bloodstream. Type I collagen consists of two α 1 and one α 2 molecules in a triple helix arrangement with the amino- and carboxy-terminal ends of the triple helix comprising a straight portion of the molecule known as a telopeptide. The telopeptide of one molecule of collagen is linked to the helical portion of an adjacent molecule by crosslinking molecules including deoxypyridinoline (DPD) and pyridinoline (PYD) (see Figure 2). Both pyridinium crosslinks can be found in other collagen tissues such as cartilage, blood vessels, or ligaments. However, they are the major crosslinks in bone with DPD occurring mainly in bone collagen. Because of the higher turnover of bone collagen compared to other collagen, both markers are considered markers for bone resorption.[3]

The amino-terminal telopeptide, carboxy-terminal telopeptide, DPD, and PYD are released into the bloodstream during resorption. They are then rapidly filtered by the kidney and released into the urine where they can be measured. One marker of bone resorption (not released from bone but from osteoclasts) is tartrate-resistant acid phosphatase isoform 5b (TRACP 5b).

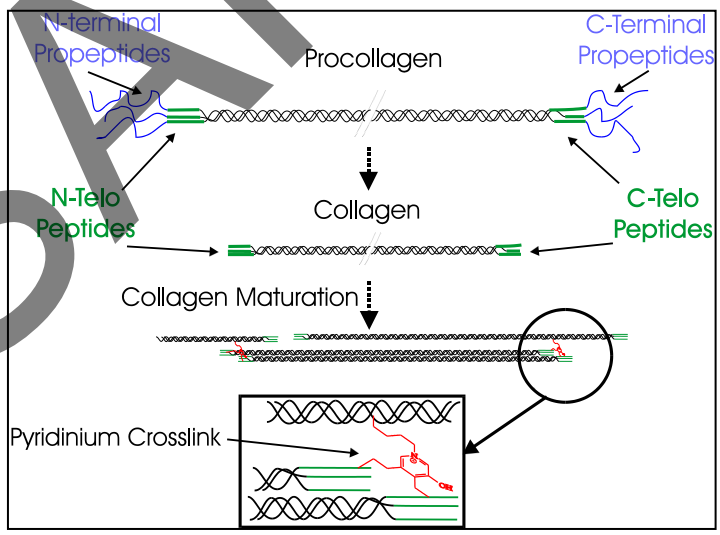

Figure 2. The Structure of Bone Collagen Depicting the Helical and Telopeptide Regions and the Crosslinks Between the Collagen Molecules. From Watts NB. Clinical utility of biochemical markers of bone remodeling. *Clin Chem.* 1999;45:1359-1368. Reprinted with permission from the American Association of Clinical Chemistry.

Markers of *bone formation* are products released by the osteoblasts such as osteocalcin and bone-specific alkaline phosphatase, or are peptides derived from the amino-terminal or carboxy-terminal of Type I procollagen, a precursor of Type I collagen. Markers of bone formation are measured in serum only. Osteocalcin is secreted by the osteoblast and incorporated into the bone matrix from which it is released during bone resorption.

Results of bone markers reported from large epidemiological or clinical trials are sometimes difficult to interpret for everyday clinical situations, i.e., the individual often has more than one disease that may affect bone turnover. Like other clinical parameters (e.g., blood lipids), analytic and biologic variability of bone markers can be significant and need to be considered when they are used.[4,5] Therefore, as with any clinical analyte, the use of bone markers requires special considerations at preanalytical, analytical, and postanalytical stages of testing. Physicians and laboratorians must understand the limitations of bone marker measurements, while appreciating their clinical utility.[6,7]

The reader is referred to a number of websites for specific guidelines on the use of biochemical markers of bone remodeling in clinical practice. A list, included as Appendix F, is not exhaustive but does acknowledge that many national and international organizations have addressed this question to varying degrees. It is recommended that the reader locate the website of the relevant osteoporosis organization in his/her country for more complete local information.

A Note on Terminology

NCCLS, 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. NCCLS 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 NCCLS, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. Despite these obstacles, NCCLS recognizes that harmonization of terms facilitates the global application of standards and is an area that needs immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In keeping with NCCLS's commitment to align terminology with that of ISO, the following describes the metrological terms and their use in C48-A:

The term *accuracy* refers to the "closeness of the agreement between the result of a (single) measurement and a true value of a measurand" and comprises both random and systematic effects. *Trueness* is used in this document when referring to the "closeness of the agreement between the average value from a large series of measurements and to a true value of a measurand;" the measurement of trueness is usually expressed in terms of *bias*. *Precision* is defined as the "closeness of agreement between independent test/measurement results obtained under stipulated conditions." As such, it cannot have a numerical value, but may be determined qualitatively as high, medium, or low. For its numerical expression, the term *imprecision* is used, which is the "dispersion of results of measurements obtained under specified conditions." In addition, different components of precision are defined in C48-A, primarily *repeatability,* i.e., "the closeness of the agreement between results of successive measurements of the same measurand carried out under the same conditions of measurement;" while *reproducibility* describes the closeness of agreement of results of measurements under changed conditions.

Key Words

Biological variability, bone marker, preanalytical variability

Application of Biochemical Markers of Bone Turnover in the Assessment and Monitoring of Bone Diseases; Approved Guideline

1 Scope

Biochemical bone marker tests are increasingly used in the management of metabolic bone diseases. They provide valuable information when performed in research centers dealing with osteoporosis and bone metabolism under well-controlled conditions and experienced personnel. However, their use in general, less specialized institutions in treating individual patients appears to be difficult,[2,8-10] and has hampered the broad use of bone markers as a clinical tool. This situation is partially due to the lack of guidance on managing and controlling biological variation and appropriate preanalytical specimen handling. Guidance is also needed for applying and using findings from clinical trials and epidemiological studies for data interpretation.

Bone markers exhibit substantial short-term and long-term fluctuations related to time of day, phase of the menstrual cycle, season of the year, diet, exercise, and other factors known to alter bone remodeling. These biological factors produce significant within-subject and between-subject variability in markers, in addition to assay imprecision and method bias. To minimize this variability, preanalytical, analytical, and postanalytical factors need to be considered and testing procedures harmonized.

This document will guide laboratorians and clinicians in selecting and using bone markers for assessing and monitoring metabolic bone diseases. Guidance is provided on managing and controlling biological variation, appropriate preanalytical sample handling, postanalytical reporting of results, and applying findings from clinical trials and epidemiological studies for data interpretation.

The document will be useful to both laboratorians and healthcare providers involved in the care of patients with bone diseases, as well as to individuals and organizations involved in developing and conducting clinical studies. In addition, this document is a starting point for discussions about further harmonization of bone marker testing.

Although efforts have been undertaken to use markers of bone metabolism in the management of related diseases such as cancer or arthritis,[8-11] such applications are not considered in this document, nor does this guideline provide information about the management of the disease with regard to medications and other forms of patient care. Such clinical guidelines need to be developed separately by the appropriate organizations.

This guideline focuses on the following bone markers:

Formation Markers

- Osteocalcin
- Total and bone alkaline phosphatase
- Type I collagen propeptides

Resorption Markers

- Pyridinoline and deoxypyridinoline
- Type I collagen telopeptides[a]
- Tartrate resistant acid phosphatase isoform 5b

[a] Comprising C-terminal crosslinking telopeptide of Type I collagen and N-terminal crosslinking telopeptides of Type I collagen.

2 Standard Precautions

Because it is often impossible to know what 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 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 U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals.* Infection Control and Hospital Epidemiology. CDC. 1996;17(1):53-80 and *MMWR* 1988;37:377-388). For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections*.

3 Definitions

A variety of abbreviations and acronyms are currently used for bone markers. To avoid misunderstandings and confusion in the literature, the subcommittee suggests the use of names and abbreviations recommended by the expert committee for the Committee of Scientific Advisors of the International Osteoporosis Foundation.[9]

Accuracy (of measurement) – Closeness of the agreement between the result of a measurement and a true value of the measurand (VIM93)[12]; **NOTE:** See the definition of **Measurand.**

Between-subject variation – Variation in analyte concentrations among individuals because of differences in factors that cannot be altered within an individual or that last for an extended period of time; **NOTE:** This includes factors such as age, sex, race, genetics, or long-term health status.

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

Bone alkaline phosphatase, bone ALP – An enzyme found in osteoblasts; **NOTES:** a) It has a molecular weight of approximately 140 000 Da; b) It is distinguishable from other alkaline phosphatases by its oligosaccharide side chain.

Bone formation – The deposition of new bone, including bone mineral and bone matrix components.

Bone marker – Biochemical substance produced or released during bone turnover; **NOTE:** It can be measured in urine, blood, or other body fluids.

Bone resorption – The process of removal of bone tissue, including bone mineral and bone matrix components.

C-terminal crosslinking telopeptide of Type I collagen, CTX – Peptides that are formed during collagen degradation, originating from the C-terminal telopeptide of collagen molecules.

Deoxypyridinoline, DPD//Lysylpyridinoline – Pyridinium compound formed during collagen maturation by crosslinking lysine and hydroxylysin side chains from different collagen molecules.

Imprecision – Dispersion of independent results of measurements obtained under specified conditions; **NOTE:** It is expressed numerically as standard deviation or coefficient of variation.

The Quality System Approach

NCCLS subscribes to a quality 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 through a gap analysis. The approach is based on the model presented in the most current edition of NCCLS HS1—*A Quality System Model for Health Care.* The quality system approach applies a core set of "quality system essentials (QSEs)," basic to any organization, to all operations in any healthcare service's path of workflow. The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Service & Satisfaction
Personnel	Process Control	Assessment	Facilities & Safety

C48-A addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the next page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
					X C28 EP14			GP29	GP27		M29

Adapted from NCCLS document HS1—*A Quality System Model for Health Care*.

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, GP26-A2 defines a clinical laboratory path of workflow which consists of three sequential processes: preanalytic, analytic, and postanalytic. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

C48-A addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the next page.

Preanalytic			**Analytic**			**Postanalytic**		
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
X		X H3 GP16	X GP16	X	X	X	X	

Adapted from NCCLS document HS1—*A Quality System Model for Health Care*.

Related NCCLS Publications*

C28-A2 **How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline—Second Edition (2000).** This document provides guidance for determining reference values and reference intervals for quantitative clinical laboratory tests.

EP14-A **Evaluation of Matrix Effects; Approved Guideline (2001).** This document provides guidance for evaluating the error or bias in analyte measurements that is due to the sample matrix (physiological or artificial) due to the sample matrix (physiological or artificial) when two analytical methods are compared.

GP16-A2 **Routine Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline—Second Edition (2001).** This guideline describes routine urinalysis test procedures that address materials and equipment, macroscopic examinations, clinical analyses, and microscopic evaluations.

GP27-A **Using Proficiency Testing (PT) to Improve the Clinical Laboratory; Approved Guideline (1999).** This guideline provides assistance to laboratories in using proficiency testing as a quality improvement tool.

GP29-A **Validation of Laboratory Tests When Proficiency Testing is Not Available; Approved Guideline (2002).** This guideline will suggest workable alternatives for evaluating the accuracy of an assay when standard interlaboratory comparison programs are unavailable.

H3-A5 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Fifth Edition (2003).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children. It also includes recommendations on order of draw.

M29-A2 **Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Second Edition (2001).** This document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

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