NCCLS Document H22-P
Vol. 4 No. 14

Proposed Standard

Histochemical Method for Leukocyte Alkaline Phosphatase

* This document is no longer being reviewed as part of the NCCLS consensus process. However, because of its usefulness to a limited segment of the clinical laboratory community, NCCLS is continuing to make the document available for its informational content.

Procedure for a semi-quantitative assay for determining leukocyte alkaline phosphatase; criteria for scoring the assay and interpreting the results.

NCCLS

This NCCLS document was published before Centers for Disease Control publication of universal precautions for the prevention of transmission of infectious disease. Universal precautions should be observed when handling specimens from all patients. See NCCLS M29-T2 *Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Second Edition*; Tentative Guideline. (Vol. 11 No. 14)

PROFESSIONS GOVERNMENT INDUSTRY

NCCLS...

Serving the World's Medical Science Community Through Voluntary Consensus

NCCLS is an international, interdisciplinary, nonprofit, standards-developing and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the healthcare community. It is recognized worldwide for the application of its unique consensus process in the development of standards and guidelines for patient testing and related healthcare issues. NCCLS is based on the principle that consensus is an effective and cost-effective way to improve patient testing and healthcare services.

In addition to developing and promoting the use of voluntary consensus standards and guidelines, NCCLS provides an open and unbiased forum to address critical issues affecting the quality of patient testing and health care.

PUBLICATIONS

An NCCLS document is published as a standard, guideline, or committee report.

Standard A document developed through the consensus process that clearly identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. A standard may, in addition, contain discretionary elements, which are clearly identified.

Guideline A document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

Report A document that has not been subjected to consensus review and is released by the Board of Directors.

CONSENSUS PROCESS

The NCCLS voluntary consensus process is a protocol establishing formal criteria for:

- The authorization of a project
- The development and open review of documents
- The revision of documents in response to comments by users
- The acceptance of a document as a consensus standard or guideline.

Most NCCLS documents are subject to two levels of consensus–"proposed" and "approved." Depending on the need for field evaluation or data collection, documents may also be made available for review at an intermediate (i.e., "tentative") consensus level.

Proposed An NCCLS consensus document undergoes the first stage of review by the healthcare community as a proposed standard or guideline. The document should receive a wide and thorough technical review, including an overall review of its scope, approach, and utility, and a line-by-line review of its technical and editorial content.

Tentative A tentative standard or guideline is made available for review and comment only when a recommended method has a well-defined need for a field evaluation or when a recommended protocol requires that specific data be collected. It should be reviewed to ensure its utility.

Approved An approved standard or guideline has achieved consensus within the healthcare community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (i.e., that comments on earlier versions have been satisfactorily addressed), and to identify the need for additional consensus documents.

NCCLS standards and guidelines represent a consensus opinion on good practices and reflect the substantial agreement by materially affected, competent, and interested parties obtained by following NCCLS's established consensus procedures. Provisions in NCCLS standards and guidelines may be more or less stringent than applicable regulations. Consequently, conformance to this voluntary consensus document does not relieve the user of responsibility for compliance with applicable regulations.

COMMENTS

The comments of users are essential to the consensus process. Anyone may submit a comment, and all comments are addressed, according to the consensus process, by the NCCLS committee that wrote the document. All comments, including those that result in a change to the document when published at the next consensus level and those that do not result in a change, are responded to by the committee in an appendix to the document. Readers are strongly encouraged to comment in any form and at any time on any NCCLS document. Address comments to the NCCLS Executive Offices, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.

VOLUNTEER PARTICIPATION

Healthcare professionals in all specialities are urged to volunteer for participation in NCCLS projects. Please contact the NCCLS Executive Offices for additional information on committee participation.

Histochemical Method for Leukocyte Alkaline Phosphatase

Message to the User

This proposed standard describes the semi-quantitative, histochemical method for determining leukocyte alkaline phosphatase (LAP). The method includes procedures for preparing fixative, buffer, and counterstains; for staining blood smears; scoring criteria for determining LAP activity; and guidance for interpreting the LAP activity score.

The subcommittee hopes that this proposed standard method will improve the reliability and interpretation of this clinically useful, widely used test.

This document is the result of much effort by the members of the Subcommittee on Cellular Enzymology. Now, we look to our membership--the clinical laboratory community--to participate in the consensus process. A questionnaire is appended to this document so that you can give us your views on the value of this project and its execution as represented by this standard. A proposed standard is the first stage in the NCCLS consensus process, and it is, therefore, especially important that we have your responses to the first three questions relating to the document's scope, utility, and scientific validity.

Your comments will help ensure that the document reflects your needs as a member of the clinical laboratory community and will help the subcommittee in related efforts in the future.

Kouichi R. Tanaka, M.D., Chairholder
Subcommittee on Cellular Enzymology

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FOREWORD

Neutrophilic leukocytes contain an alkaline phosphomonesterase with variable activity in normal subjects and in patients with hematological and nonhematological disorders. Leukocyte alkaline phosphatase is a well established term and thus will be used, although we recognize that in the peripheral blood the enzyme is virtually restricted to neutrophil polymorphonuclear cells.

There is no relationship between leukocyte alkaline phosphatase and serum alkaline phosphatase. The function of leukocyte alkaline phosphatase is still unknown, but normal leukocyte alkaline phosphatase depends on an intact pituitary adrenal axis.

Clinical interest in leukocyte alkaline phosphatase was stimulated initially by the observation that the leukocytes of patients with chronic granulocytic leukemia usually had low levels of enzymatic activity in contrast to high values seen in leukocytosis of other disorders. Quantitative methods of assay are available but are not practical. Semiquantitative assay by a variety of azo dye histochemical methods has made determination of leukocyte alkaline phosphatase a widely used clinical test. This document describes a standard histochemical method which should serve as a benchmark.

KEY WORDS

Leukocyte alkaline phosphatase (LAP); phosphatase activity; LAP, histochemical method; granulocytic leukemia; leukocytosis; LAP, scoring criteria.

HISTOCHEMICAL METHOD FOR LEUKOCYTE ALKALINE PHOSPHATASE

1.0 PURPOSE

Estimating leukocyte alkaline phosphatase activity is most useful clinically in differentiating chronic granulocytic leukemia from leukocytosis as seen in severe infections, polycythemia rubra vera, and myelofibrosis with myeloid metaplasia.

2.0 PRINCIPLE

The substrate is hydrolysed by enzymatic activity at pH 9.5 releasing phosphate and an aryl naptholamide. The aryl naphtholamide immediately couples with the diazonium salt present in the incubation mixture forming an insoluble azo dye at the theoretical sites of enzyme activity.

3.0 APPARATUS

Routinely used, clean glassware

4.0 REAGENTS

(1) naphthol AS-BI phosphate, sodium salt

(2) fast violet B salt (purified), 6-benzamido-4-methoxy-m-touidine, diazonium chloride

(3) 2-amino-2-methyl-1,3-propanediol

(4) 37% formaldehyde

(5) absolute methanol

(6) absolute acetone

(7) 0.1 N hydrochloric acid

(8) hematoxylin powder

(9) sodium iodate

(10) aluminum potassium sulfate, $A1K(SO_4)_2 * 12 H_20$

(11) 0.03 M sodium citrate, $Na_3C_6H_5O_7 * 2 H_20$ - (4.41 g/500 mL H_2O)