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November 1999

C37-A

Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline

This guideline details procedures for the manufacture and evaluation of human serum pools for cholesterol measurement.

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

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Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: +1.610.688.0100
F: +1.610.688.0700
www.clsi.org
standard@clsi.org

Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline

Abstract

Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline (C37-A) outlines procedures for selecting, processing, and combining donor units to prepare frozen serum pools which are commutable among multiple methods for serum cholesterol measurement. The guideline also addresses issues related to the evaluation of the pooled materials. The manufacture and evaluation of two levels of serum cholesterol pools prepared according to this guideline are described. The appendix includes a summary of the results from the pilot study conducted to evaluate the scientific basis of this guideline.

This guideline will provide information to develop reference materials which can be useful to manufacturers of *in vitro* diagnostic (IVD) reagents, systems, and quality control products in establishment of calibration (or assigned values) for their products. Additionally, these reference materials can be beneficial to proficiency testing agencies, as well as end users in the clinical laboratory as tools to independently assess performance (and trueness) of procedures for the routine measurement of serum cholesterol.

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Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline

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Gary L. Myers, Ph.D., Chairholder
John H. Eckfeldt, M.D., Ph.D.
Neil Greenberg, Ph.D.
Jacob B. Levine, M.B.A.
W. Gregory Miller, Ph.D.
Donald A. Wiebe, Ph.D.



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Foreword

Numerous epidemiological and clinical investigations across the world have documented that elevated levels of blood cholesterol are a major risk factor for coronary heart disease (CHD) and that lowering blood cholesterol levels decreases morbidity and mortality from CHD.¹⁻⁵ Reduction in the prevalence of elevated blood cholesterol has been a primary objective of the National Cholesterol Education Program (NCEP), established in 1985 by the National Heart, Lung and Blood Institute.^{6,7} The efforts of NCEP and other similar international cholesterol education programs to reduce high blood cholesterol are based on the need for reliable blood cholesterol measurements for proper patient risk classification.⁸ An important milestone in the NCEP effort was the establishment of the National Reference System for Cholesterol (NRS/CHOL) by NCCLS, which provides the accuracy target for cholesterol measurement.⁹ The NCEP's Laboratory Standardization Panel recommended that all cholesterol measurements be standardized and traceable to the NRS/CHOL.⁸ It is extremely important that manufacturers of clinical instruments, reagents, and calibrator materials, as well as clinical laboratories, have available to them reference materials that will establish traceability to the NRS/CHOL and assure true and precise results on human subjects, in all field methods being used worldwide. In order to achieve this goal, reference materials with long-term stability and appropriate analyte concentrations are required.

An additional very important requirement is commutability, termed "transmutability" by some investigators. Even though often prepared from human sources, processed reference materials in the past have often taken on properties, loosely termed "matrix effects," that make them behave differently from patient specimens in some reagent/instrument systems.¹⁰ Matrix effects differ from more typical analytical interferences in that the latter are effects of exogenous (e.g., drug) or endogenous (e.g., bilirubin) substances that have similar effects on both patient and manufactured control and calibrator materials. In contrast, matrix effects are properties that are unique to the processed reference materials (and control materials). Since matrix effects lead to underrecovery, or less commonly overrecovery, of the analyte of interest in some field methods, knowing very true and precise analyte concentrations on a reference material which demonstrates matrix effects with certain field methods does little to transfer trueness to those methods.

The currently accepted approach to document traceability to the NRS/CHOL for many field methods is via a split-sample comparison with the reference method using fresh human serum specimens.¹¹ The presence or absence of matrix-effect interferences with field methods in a given sample can be confirmed by direct comparison of field method measurements with measurements from a reference method which is not subject to matrix effects. Protocols have been developed which can identify matrix effects in particular materials.¹² However, the use of commutable reference materials is the preferred approach and, therefore, a need exists to develop reference materials for cholesterol measurement procedures that do not demonstrate matrix effects. The few materials-formulation guidelines that exist are of a generic nature, not specifically addressing the problem of modern cholesterol methods and the newer, high-throughput, microsampling analytical equipment.¹³⁻¹⁵ It is therefore clear that there is a significant gap in the NRS/CHOL and that an immediate need exists for agreement on material standards applicable to the establishment of the calibration and trueness of serum cholesterol measurement systems.

Key Words

Cholesterol, commutable, matrix effects, NRS/CHOL, reference materials, serum pools, traceability

Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline

1 Introduction

This guideline describes the procedures for preparing frozen human serum pools of high quality suitable for cholesterol measurement. Although prepared for cholesterol, the concepts described in this written guideline may be applied to the preparation of frozen human serum pools for other analytes.

2 Scope

These specifications are designed to enable laboratory scientists, *in vitro* diagnostic (IVD) manufacturers, proficiency testing providers, and suppliers of clinical laboratory reference materials^a to prepare frozen human serum pools which demonstrate minimal matrix effects, are commutable across different cholesterol measurement procedures, and, as such, are suitable for assessing the trueness of these procedures. These serum pools, when assigned target values by the definitive method and/or reference method of the National Reference System for Cholesterol (NRS/CHOL), may be used to properly calibrate and/or assess trueness of field methods for serum cholesterol measurement, and provide an alternative method to split-sample comparisons with human specimens for establishing traceability of calibration of field methods to the NRS/CHOL. This document provides guidance for collecting and processing raw materials to manufacture frozen serum pools and for performing quality assurance of the final product. As part of the guideline development, two frozen human serum pools using a large number of selected individual donor units of "off-the-clot" serum were prepared and characterized. The performance of the pools was assessed in terms of the degree of commutability (observed response versus predicted response) relative to the individual sera which comprised the pools.

^a Examples of suppliers of reference materials in the U.S are the National Institute of Standards and Technology, the College of American Pathologists, and the Centers for Disease Control and Prevention.

3 Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to "standard precautions." Standard precautions are new guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution 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, Vol 17;1:53-80.), [MMWR 1987;36(suppl 2S):2S-18S] and (MMWR 1988;37:377-382, 387-388). For 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, refer to NCCLS document M29—*Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*.

4 Definitions^b

Accuracy, *n* - Closeness of the agreement between the result of a measurement and a true value of the measurand. **NOTE:** The concept of accuracy of measurement is described by trueness of measurement and precision of measurement. Thus, accuracy is not a synonym for trueness or for precision (VIM: 1993, 3.5¹⁶).

Aseptic, *adj* - Environmental conditions which minimize microbial contamination.

^b Some of these definitions are found in NCCLS document NRSL8—*Terminology and Definitions for Use in NCCLS Documents*. For complete definitions and detailed source information, please refer to the most current edition of that document.

Commutable, *adj* - Interassay properties of a reference material, calibrator material, or quality control material that are comparable with those demonstrated by authentic clinical specimens.¹⁷

NOTE: Commutability of a material is defined as the "degree to which a material yields the same numerical relationships between results of measurements by a given set of measurement procedures, purporting to measure the same quantity, as those between the expectations of the relationships obtained when the same procedures are applied to other relevant types of material" (CEN prEN 12287:1999, 3.5¹⁸).

Hemolysis, *n* - Serum appearing red or pink in color resulting from the breakdown of red blood cells liberating hemoglobin.

Homogeneity, *n* - The condition of being of uniform structure or composition with respect to one or more specified properties.

Icterus, *n* - Serum with yellow color resulting from elevated bilirubin in the blood.

Lipemia, *n* - A milky or turbid serum resulting from elevated lipids in the blood.

Matrix effect, *n* - The influence of a component in the sample, other than the analyte, on the measurement, and thereby on the value, of the measurable quantity.

Off-the-clot serum, *n* - The liquid remaining after neat whole blood has coagulated and the clot has retracted and/or been spun down in a centrifuge to separate the clot from the liquid portion.

Reference material, *n* - A material or substance, one or more of whose property values are sufficiently homogeneous and will be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials (ISO Guide 30:1992, 2.1¹⁹), (VIM: 1993, 6.13.¹⁶). **NOTE:** A given reference material may be used either as a calibration material in calibration of a measuring system and in assigning values to materials, or as a control material in assessing the performance of a measurement procedure, but must not be used for both purposes in a given situation in a particular laboratory.

Traceability, *n* - The property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons, all having stated uncertainties.

Trueness, *n* - Closeness of agreement between the average value obtained from a large series of results of measurements and a true value.

NOTES: a) Trueness of measurement cannot be given a numerical value, but can be expressed on an ordinal scale such as (low, medium, high); b) Trueness is usually expressed numerically by the statistical measure bias that is inversely related to trueness. (ISO 3534-1:1993²⁰), (ISO 5725-1:1994²¹). See also **Accuracy**.

5 Design Considerations

5.1 Pool Preparation

During the deliberations that took place to develop this guideline, a number of issues that could significantly impact the quality of the final product were considered. The most important of these issues are presented briefly here.

The collection conditions used for the individual units are designed to provide off-the-clot serum with minimal degradation or modification during handling and processing. Completion of blood collection and processing, dispensing, and freezing must be done as quickly as possible to assure the quality of the final product.

A major assumption made when preparing serum pools by combining donor units is that the behavior of the pooled sera is not adversely affected by the influence of any human sample-specific biases. The effect of an interference that might occur from a donor can be estimated by assuming that bias is proportional to the donor's serum volume contribution to the pool. If this assumption is correct, the bias introduced into the pool is the bias of the single-donor divided by the total number of donors ($\text{Bias}_{\text{pool}} = \text{Bias}_{\text{donor}}/N$). For example, if a single-donor sample had a large cholesterol bias of 30% due to interferences, a 1-% bias in a pool comprised of 30 donors would result. Therefore it is important to use a sufficient number of donors in order to minimize the effect any single donor might have on the pool.

Related NCCLS Publications*

- M29-A** **Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline (1997).** 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.
- NRSCL8-A** **Terminology and Definitions for Use in NCCLS Documents; Approved Standard (1998).** This document provides standard definitions for use in NCCLS standards and guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

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

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

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