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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.



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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.

NCCLS. *Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline*. NCCLS document C37-A (ISBN 1-56238-392-2). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 1999.

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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.

Collection into plastic blood bags is recommended as an industry standard procedure. However, plastic blood collection bags are suspected of contaminating serum with phthalate esters and other plasticizer chemicals, depending on the length of contact time with the plastic. It is believed that serum left in contact with plastic blood collection bags for extended periods will alter the lipoproteins and thus adversely affect the biochemical stability of the pool. Collection with the blood bag in ice water prevents clotting to allow rapid separation of plasma from cells. Rapid separation of red blood cells from plasma prior to clotting minimizes exposure to cells and plastic to avoid biochemical processes and contamination from plastic-derived molecules. Centrifugation conditions for the cold blood bags were chosen to produce plasma with adequate platelets for clotting after aseptic transfer into glass bottles. Plasma is transferred to a sterile glass bottle for clotting to minimize the time exposed to plastic surfaces. Evacuated bottles should not be used to receive the plasma, because flow through the tubing must be controlled with a pinch valve to prevent frothing of the plasma which can introduce an undesirable random variable in the process.

The two pools prepared to validate this guideline were evaluated for the presence of plasticizer chemicals. The toxicology laboratory at the University of Wisconsin Hospital and Clinics (UWHC), Madison, Wisconsin, performed a qualitative assessment of these two serum pools by GC/MS to detect the presence of plasticizers. Two additional samples were included in the evaluation: one sample obtained from the UWHC Blood Bank (outdated unit of autologous frozen serum stored in plastic), and the second was drug-free serum prepared in-house from material collected and stored in glass. Cholesterol (free) was the predominant peak noted in each sample tested, along with a series of peaks for free fatty acids. It is of interest to note that the free fatty acids of the two pools prepared by these guidelines were minor compared to the free cholesterol suggesting minimal disruption of the lipid and lipoprotein characteristics of the original donors. A major peak that has the characteristics of phthalate esters typically found in plasticizers was detected in the blood bank specimen. This peak was of minor consequence in the two pools prepared according to this guideline. The

impact of trace amounts of phthalate esters present in the materials was not evaluated as part of this project.

Aseptic handling wherever possible will minimize exposure to potential microbial contamination, and relatively short times at ambient temperature minimizes bacterial growth in the event of contamination. Glass containers used during the blood processing steps were sterilized by autoclaving to prevent bacterial contamination.

Borosilicate glass containers with Teflon[®]-lined caps and stoppers should be used for processing serum and for storage vials. These materials are selected to minimize contamination from surface interactions and chemical leaching.

Membranes for filtration of the serum pool should be selected to minimize adsorption of proteins or other serum constituents. The membranes should be tested for suitability by measuring critical analytes before and after a trial filtration. Polyvinylidene difluoride membranes meet these requirements. Membranes should be washed appropriately to avoid contamination with preservative materials.

Individual serum units are not filtered prior to pooling, in order to match as closely as possible the matrix usually used for testing in a clinical laboratory, and to avoid increased risk of bacterial contamination. Not filtering individual units also minimizes the time between collection of blood and freezing of the pooled sera.

It is desirable to avoid shipping serum units from a collection center to a pool preparation center. Shipping introduces a random variable into the process which has the potential for degradation of the serum. If shipping cannot be avoided, the serum containers must be 100% filled with no air space. This condition minimizes the opportunity for mechanical agitation and denaturation of protein and other constituents. Shipping conditions must be adequate to maintain temperature of all material at ≤ 8 °C.

Freezing of the pooled sera is critical. Freezing conditions should be uniform for each vial. A maximum temperature of -70 °C should be

achieved for storage of the frozen sera. This temperature is necessary for complete ice formation and immobilization of water molecules in the presence of the serum matrix constituents. Complete ice formation limits molecular diffusion and interactions which can result in degradation of lipoproteins and other molecules over time.

Following initial freezing, vials must be handled for transfer or shipping in such a way that they do not experience greater than one hour cumulative between -70 to -30 °C. Any vial in which the contents become partially or fully liquefied must be immediately used or discarded. **Material should not be refrozen.**

5.2 Validation of Performance and Commutability

Commutability of the prepared reference material must be demonstrated through an appropriate validation procedure. Commutability of a reference material is demonstrated when the reference material shows intermethod changes in response, per measurement unit, comparable to the changes observed for native individual human serum samples. Validation of the commutability of a reference material is therefore demonstrated when the reference material yields a consistent numerical or ratiometric relationship for the among-methods results, when compared to the among-methods numerical relationship (for the same measurement methods) established with the relevant routine test samples (i.e., freshly collected individual donor human serum.)

For the reference materials prepared to verify this written guideline, aliquots of the original individual donor serum units were retained and set aside before pooling for later use in the validation studies. An alternative approach which may be considered is use of an independent series or panel of selected individual human specimens, employing donor selection criteria as outlined in this written guideline. The advantage of the first approach (retained aliquots of the original donor units) is that it also permits an evaluation to rule out the effects of pooling on the performance of the reference materials. The results obtained with the pooled reference materials should be fully predictable from the mathematical summation and averaging of the results for the individual

donors, if there is no significant pooling effect. The second approach (independent series or panel of specimens) may be preferred, since an interference imparted to the pool by a single donor is sometimes not linear after dilution with sera of other donors. Lack of predictability of interferences underscores the importance of performing commutability verification studies with each pool, as part of a routine quality assurance process, prerequisite to releasing a given pool for use as a reference material.

With either approach to validation, the protocol is essentially a series of method comparison studies, which includes the candidate reference materials, tested in parallel with the panel of individual human serum samples. Although a full panel of fresh human serum samples is preferred, it may not be practical to obtain the range of samples appropriate to cover the reportable range of the method/procedure in a time frame that would support such a study. In a situation where samples need to be preserved to support validation studies of performance and commutability, careful storage of the samples at -70 °C is required. Analysis of the data focuses on the establishment of the intermethod numerical relationships, determined separately for the individual donor samples and the candidate reference materials. To demonstrate commutability, the calculated ratios, or numerical relationships obtained for these separate comparisons (method-to-method for a panel of individual donor specimens), should be statistically equivalent.

For more detail on specific analytical methodology for the statistical comparisons required, the papers by Eckfeldt¹² and Long²² should be consulted. Additional details of the validation studies for the reference materials described in this guideline are found in [Appendix B](#).

6 Raw Materials

6.1 Donor Selection

Donors should be selected according to accepted criteria and local governmental regulations.^c An informed consent must be

^c For example, *Technical Manual of the American Association of Blood Banks* (AABB, Arlington, VA 22209).

obtained in writing from each donor after the specific collection procedure is explained and the donor is cautioned as to possible risks associated with blood donation. As part of the informed consent, donors should be informed of any tests performed on blood collected, the availability of results to donors, and the end use of blood collected. Adequate safeguards for donor privacy must be ensured. Donors should be prescreened and segregated according to desired concentration levels and number of pools required. They should fast for a minimum of eight hours prior to blood collection.

6.2 General Exclusion Criteria for Donor Units and Pools

Serum units and/or pools (as indicated) should be tested and accepted if the following specifications are met:

- Each unit—Should be negative for antibodies to human immunodeficiency virus, hepatitis B surface antigen, and antibody to hepatitis C virus.
- Each unit—For serum cholesterol and triglycerides, units outside of needed ranges should be rejected. “Spiking” to achieve cholesterol and triglyceride levels is specifically prohibited.
- Each unit and pool—Absence of clotting elements. To test for this, heat an aliquot of serum to 55 °C for five minutes. Turbidity measured at 710 nm in a 1-cm cuvette should remain constant after heating (± 0.03 A).
- Each unit and pool—OD < 0.5 at 710 nm in a 1-cm cuvette.
- Each unit—Bilirubin < 1.5 mg/dL (25.6 μ mol/L).
- Each unit—Total protein 5.8 to 8.0 g/dL (58 to 80 g/L).
- Each unit—Hemoglobin < 30 mg/dL (0.3 g/L).
- Each unit—Should be visually clear, straw-, or yellow-colored and free from particulate matter.
- Each unit and pool—pH = 7.4 to 8.2.
- Each unit and pool—Albumin/globulin ratio > 1.0.
- Each unit and pool—Glucose > 60 mg/dL (3.33 mmol/L). “Spiking” to achieve glucose levels is specifically prohibited.
- Pool—Ammonia: Target to < 100 μ mol/L. The pool should be rejected if ammonia exceeds 150 μ mol/L.
- Pool—Microbial contaminants: Standard agar cultures must yield growth of < 10 CFUs per mL of pooled serum.
- Pool—Additional microbial testing must be performed to specifically rule out the presence of the following pathogenic organisms: *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.
- Pool—Homogeneity. Test the final pool(s) to assess uniformity of all vials relative to cholesterol concentration, per Appendix A.

7 Blood Collection and Processing Procedure

Refer to [Appendix B3.1](#) for details on materials and apparatus suitable for use with the written guideline. Items can be used which are equivalent to those used in procedures reported here.

- (1) Collect, in an ice-water bath, individual donor units of whole blood using an industry-standard empty blood-pack with integral donor tube, a 15-gauge needle, and a 600-mL plastic container.
- (2) Within five minutes of unit collection, before unit clots, separate plasma from cells by centrifugation at 1,500 x g for eight minutes at 4 °C.
- (3) Aseptically express plasma into a clean and sterile 250-mL borosilicate glass bottle with a Teflon®-lined screw cap. Avoid using vacuum bottles. Control plasma flow into the transfer bottle to avoid spattering and frothing.
- (4) Allow clotting to proceed at room temperature until clot retracts fully from

- the serum. To maximize collection yield, clotting time should be three to four hours.
- (5) Centrifuge each unit at 2,700 x g for at least 19 minutes.
 - (6) Aseptically transfer clear serum layer to a clean and sterile 250-mL borosilicate glass bottle with Teflon[®]-lined screw cap. Test for absence of clotting elements (per [Section 6.2](#), third bullet). If negative, proceed with Step 7. If positive, repeat Steps 4 to 6, one additional cycle. If positive result persists, reject unit.
 - (7) Exclude lipemic, icteric, or hemolyzed serum units. Perform tests for attributes (see [Section 6.2](#)). Units failing any of these tests should be rejected from the pool.
 - (8) Store all units at 4 °C, and maintain chilled until pooling. Do not freeze. If transportation is required at this stage, the method should avoid agitation of the liquid.
 - (9) Prior to pooling (after storage at 4 °C), visually inspect each unit to assure that it is clear, straw- or yellow-colored liquid, free of particulates.
 - (10) Prepare the serum pool using the serum from the donor units (units selected for inclusion in a pool based on specific analyte level desired).
 - (11) Let pool incubate at 4 °C for 18 hours with constant, low-speed, magnetic stirrer mixing to assure homogeneity of the final pool and to allow any interactions to occur between molecules from individual units.
 - (12) Sterile filter pool through a 0.22 µm absolute filter (hydrophilic membrane). Collect filtrate into an appropriate sized sterile glass flask prior to final dispensing of pool (add sterile Teflon[®] stir bar).
 - (13) Aseptically transfer desired volume aliquots of pooled serum to final containers and stopper and seal. Maintain pool at 4 °C on ice with constant stirring to assure homogeneity of product throughout the dispensing process.
 - (14) Uniformly freeze the pool. Do not use Styrofoam racks or boxes which may cause freezing gradients from the outside to the inside of the container. All vials must be frozen in an upright position. Place all sample boxes in the freezer at the same time, being sure that each box is in direct contact with the metal shelf of the freezer.
 - (15) Store the filled containers at -70 °C.
 - (16) Blood collection, processing, dispensing, capping, crimping, freezing, and storage of the pool should be completed within 56 hours.

References

- 1 Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Trial Results, I: reduction in incidence of coronary heart disease; II: the relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA*. 1984;251:351-374.
- 2 Frick MH, Elo O, Haapa K, et al. Helsinki Heart Study: primary prevention trial with gemfibrozil in middle-aged men with dyslipidemia. *N Engl J Med*. 1987; 317:1237-1245.
- 3 Stamler J, Wentworth D, Neaton JD, for the MRFIT Research Group. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? *JAMA*. 1986;256:2823-2828.
- 4 Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet*. 1994;344: 1383-1389.
- 5 Shepherd J, Cobbe SM, Ford I, et al., for the West of Scotland Coronary Prevention Study Trial. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med*. 1995;333:1301-1307.
- 6 Lenfont C. A new challenge for America: The National Cholesterol Education Program. *Circulation*. 1985;73:855-856.
- 7 Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. *Arch Intern Med*. 1988;148:36-39.
- 8 Current status of blood cholesterol measurement in clinical laboratories in the United States: A report from the Laboratory Standardization Panel of the National Cholesterol Education Program. *Clin Chem*. 1988;34:193-201.
- 9 NCCLS. *Approved Summary of Methods and Materials Credentialed by the Council of the NRSC*. Document RS3-A. Villanova, PA: NCCLS;1987.
- 10 Miller WG. Matrix effects in the measurement and standardization of lipids and lipoproteins. In: Rifai N, Warnick GR, and Dominiczak MH, eds. *Handbook of Lipoprotein Testing*. Washington, D.C.: AACC Press, 1997;199-221.
- 11 Recommendations for improving cholesterol measurement: A report from the Laboratory Standardization Panel of the National Cholesterol Education Program. NIH Publication No. 90-2964. Bethesda, MD: NIH, February 1990.
- 12 Eckfeldt JH, Copeland KR. Accuracy verification and identification of matrix effects. The College of American Pathologists' Protocol. *Arch Pathol Lab Med*. 1993;117:381-386.
- 13 Williams JH, Taylor L, Kuchmak M, Witter RF. Preparation of hypercholesterolemic and/or hypertriglyceridemic sera for lipid determinations. *Clin Chim Acta*. 1970;28:247-253.
- 14 Proksch GJ, Bonderman DP. Use of a cholesterol-rich bovine lipoprotein to enhance cholesterol concentrations in the preparation of serum control materials. *Clin Chem*. 1976;22:1302-1305.
- 15 Kuchmak M, Taylor L, Williams JH. Preparation of reference sera with desired levels of cholesterol and triglyceride. *Clin Chim Acta*. 198;114: 127-135.
- 16 International Organization for Standardization (ISO). *International Vocabulary of Basic and General Terms in Metrology (VIM)*. Geneva; 1993.

- 17 Fasce CF, Rej R, Copeland WH, Vanderlinde RE. A discussion of enzyme reference materials: Applications and specifications. *Clin Chem*. 1973;19:5-9.
- 18 European Committee for Standardization (CEN). In vitro diagnostic systems — Measurements of quantities in samples of biological origin — Presentation of reference measurement procedures (draft). Ref. No. prEN 12287: 1999. Central Secretariat: rue de Stassart 36, B-1050 Brussels, Belgium.
- 19 International Organization for Standardization (ISO). *Terms and definitions used in connection with reference materials*. Geneva:1992; ISO Guide 30.
- 20 International Organization for Standardization (ISO). *Statistics Vocabulary and Symbols*. ISO 3534-1:1993. Geneva: 1993;ISO 3534-1.
- 21 International Organization for Standardization (ISO). *Accuracy (trueness and precision) of measurement methods and results*. Geneva:1994;ISO 5725-1.
- 22 Long T. Statistical power in the detection of matrix effects. *Arch Pathol Lab Med*. 1993;117:387-392.

Appendix A. Quality Control Sampling Test for Pool Homogeneity

A1 Test Objective

Assure that serum pools prepared according to guideline demonstrate uniformity across the production lot for cholesterol concentration.

A2 Test Assumptions

Cholesterol homogeneity testing is to be conducted with a cholesterol method which has a within-run coefficient of variation of $\leq 1.5\%$. The vendor should confirm the within-run coefficient of variation for the method of choice.

A3 Test Plan

- (1) Select one vial from each of a minimum of 15 different periods spaced in time equally throughout the dispensing/ aliquoting run.
- (2) For each pool, perform in a single run, quadruplicate cholesterol analyses on each of the 15 sample vials. This design will allow an analysis of variance to be run to check for significant vial-to-vial variability within periods of the dispensing run and to check for significant vial-to-vial variability over the entire dispensing run.

A4 Data Analysis

- (1) Analyze the data using a one-way analysis of variance (ANOVA). The analysis of variance table will appear as follows:

Source of Variation	DF	Expected Mean Square
Among vial	14	$(S_a)^2 + (4)(S_v)^2$ (A)
With-in vial	45	$(S_a)^2$ (B)

where $(S_a)^2$ is the analytic variance, $(S_v)^2$ is the vial-to-vial variance [i.e., the heterogeneity of the pool], and DF is the degrees of freedom associated with the mean square estimate. Four is the number of replicate determinations per vial.

- (2) Calculate the F-ratio formed by (Mean Square A)/(Mean Square B) with 14 and 45 degrees of freedom. The critical value of F (probability = 0.05) for this comparison is 1.918. If the calculated F-ratio is greater than 1.918, the vial-to-vial variation for the pool may be too large and therefore the pool should be evaluated further to substantiate the homogeneity of the pool before considering rejection.

NOTE: This procedure provides a test of the null hypothesis that the vial-to-vial variance is zero (i.e., the pool is perfectly homogenous) versus the alternative hypothesis that the vial-to-vial variance is greater than zero (i.e., the pool is not perfectly homogeneous). The sample size (i.e., the number of vials and the number of measurements per vial) was chosen so that the F-test would have probability of 0.80 or greater of rejecting a pool with a vial-to-vial coefficient of variation $\geq 1\%$. In order for the F-test to have the stated power, it is very important that the within-run coefficient of variation be no larger than 1.5%.

Appendix B. Verification of Material Specifications

To validate the written guideline, the subcommittee had prepared, according to the material specifications, two frozen serum pools using a large number of selected individual donor units of "off-the-clot" serum. The performance of the pools was assessed in terms of the degree of commutability (true response versus predicted response) relative to the individual sera which comprise the pools. This section describes the preparation and evaluation of the donor units and pools (Figure B1) and the study conducted to validate the guideline.

B1 Validation Study Materials

Two pools of off-the-clot human serum (combined per cholesterol level within specified ranges) were prepared, packaged in 1-mL aliquots (each contained in a 3-mL glass vial), labeled, and frozen. Sixty 1-mL aliquots (each contained in a 3-mL glass vial), uniquely labeled and traceable to the original donor unit, were collected from each of the approximately 80 individual donor units (44 for Pool 1 and 43 for Pool 11).

B2 Donor Selection

B2.1 Criteria

Donors were selected according to selection criteria in the *Technical Manual of the American Association of Blood Banks*.^d Donors were prescreened for serum cholesterol concentration and divided into two groups.

Recommended analyte ranges for two cholesterol pools were: cholesterol 160 to 230 mg/dL (4.14 to 5.96 mmol/L) with triglycerides <200 mg/dL (2.26 mmol/L); and cholesterol 240 to 380 mg/dL (6.22 to 9.84 mmol/L) with triglycerides <300 mg/dL (3.39 mmol/L). Selected donors were asked to fast a minimum of eight hours.

Donors were recruited based on previous history of lipid values and their willingness to provide a fasting unit of whole blood. Donors with cholesterol concentrations of less than 200 mg/dL (5.18 mmol/L) and triglyceride concentration of less than 200 mg/dL (2.26 mmol/L) were scheduled for collection during the morning hours of two consecutive days for preparation of the low/normal-lipid pool. A week later individuals with lipids expected in the range of 260 to 350 mg/dL (6.73 to 9.06 mmol/L) cholesterol and triglycerides less than 300 mg/dL (3.39 mmol/L), were again scheduled for two consecutive days to prepare the high lipid pool. A minimum of 40 donors would be required for each pool preparation; however, 52 individuals were processed for the low/normal-lipid pool and an additional 58 donors were recruited for the higher pool to account for any individual units that might fail to meet the criteria.

^d *Technical Manual of the American Association of Blood Banks* is available from AABB, Arlington, VA 22209.

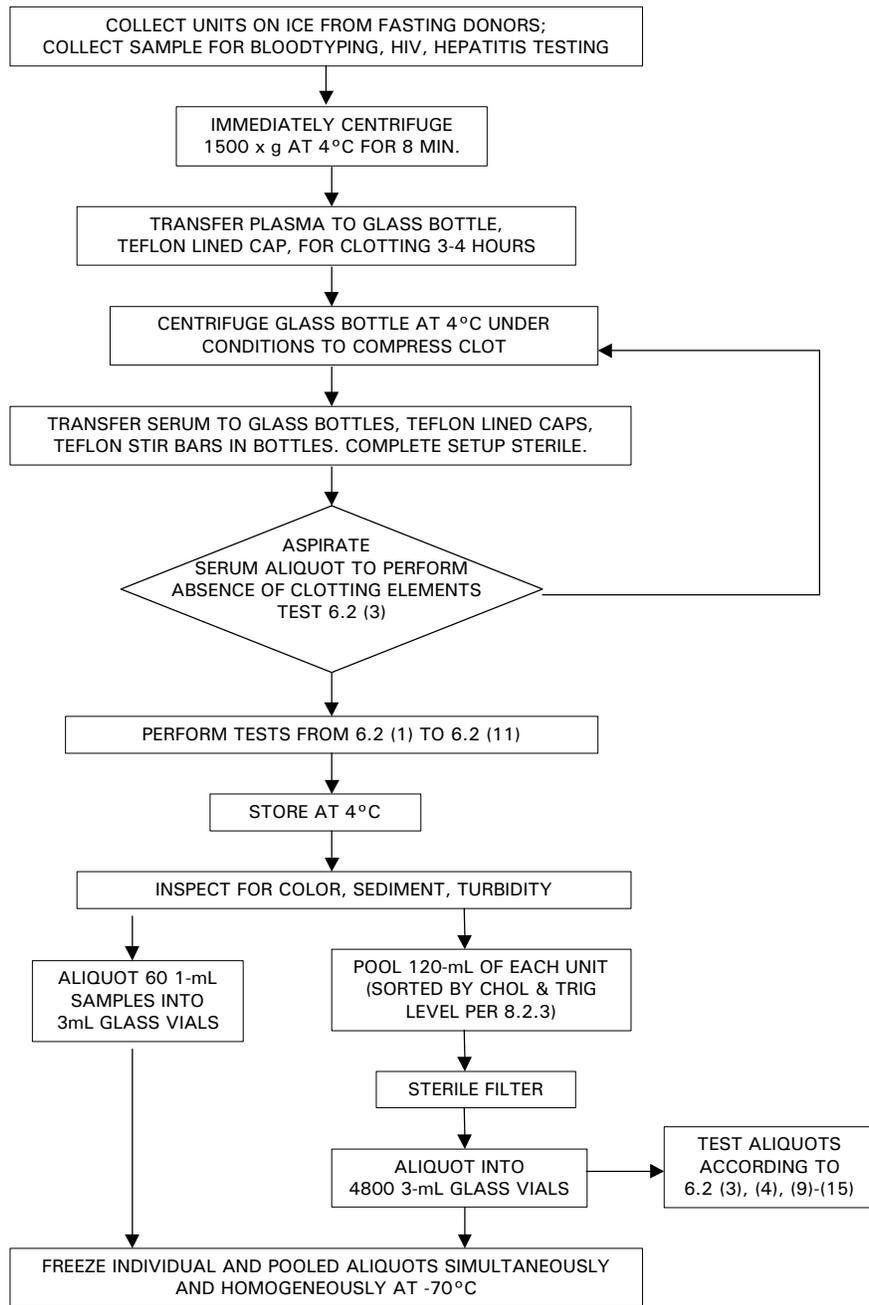


Figure B1. Preparation and Evaluation of the Donor Units and Pools

B2.2 General Exclusion Criteria for Individual Units

- All HbsAg, HIV Ab, and HCV Ab positive serum units
- Serum units that failed routine clinical test criteria as follows:
 - a. Total Protein 5.8 to 8.0 g/dL (58 to 80 g/L)
 - b. Optical density < 0.5 at 710 nm
 - c. Bilirubin < 1.5 mg/dL (25.6 μ mol/L)
 - d. Hemoglobin < 30 mg/dL (0.3 g/L)
 - e. pH = 7.4 to 8.2
 - f. A/G ratio > 1.0
 - g. Glucose > 60 mg/dL (3.33 mmol/L)
 - h. Absence of clotting factors
 - i. Desired group lipid characteristics

B2.3 Cholesterol and Triglyceride Acceptance Criteria

Pool	Cholesterol	Triglyceride	Appearance
Group A	180 to 200 mg/dL (4.66 to 5.18 mmol/L)	< 200 mg/dL (2.26 mmol/L)	no turbidity
Group B	260 to 350 mg/dL (6.73 to 9.06 mmol/L)	< 300 mg/dL (3.39 mmol/L)	no turbidity

B3 Materials and Apparatus

B3.1 Apparatus

- Whole blood collection kits with 600-mL capacity, Teruflex B-600 code BB, Terumo Corp., Tokyo, Japan.
- Refrigerated centrifuge equipped with six-place rotor TY-JS4.2 and six carriers (339127), Model Beckman J6B.
- Glass bottles (200 mL) with Teflon[®]-lined screw caps (210-25-010), VWR Scientific.
- Refrigerated centrifuge equipped with a four-place rotor H1008 and single-slot carriers (250), Model Sorvall RT6000, DuPont.
- Sterile filters with 0.22- μ m hydrophilic membranes, KVGL 04HB3, Durapore[®] Millipore.
- Pressurized stainless steel tank with a 5-gal. (18.9-L) capacity (RG5), Amicon, Inc., Beverly, MA.
- Borosilicate, 250-mL capacity, serum bottles with Teflon[®]-lined screw caps (219817); 3-mL borosilicate clear "400" glass vials (223715); 13-mm gray Teflon[®]-coated stoppers (1012-4620); and center tear-out aluminum crimp seals (224182-01), Wheaton Science Products, Millville, NJ.
- Foot-controlled dispenser, Unispense (374301) and an air-powered crimper (224382), Wheaton Science Products, Millville, NJ.
- Hitachi 705 chemistry analyzer, Boehringer Mannheim Corporation, Indianapolis, IN.
- Model DU-20 spectrophotometer, Beckman Instruments, Fullerton, CA.

B3.2 Reagents

- Reagents for HbsAg, HIV Ab, and HCV Ab testing were purchased from Abbott Laboratories, Abbott Park, IL.
- Tetramethylbenzidine was purchased from Aldrich Chemical Company, Milwaukee, WI.
- Hydrogen peroxide was obtained from J.T. Baker Chemical Company, Phillipsburg, NJ.
- BBL media was obtained from Becton Dickinson Company, Franklin Lakes, NJ.
- Other reagent grade chemicals or highest possible purity were acquired from various manufacturers.

B4 Blood Collection and Processing

Donors received venipuncture with a 15-gauge needle and whole blood was collected into an empty (no preservatives nor anticoagulants) 600-mL capacity plastic bag that was placed in an ice bath to retard clot formation. A second blood specimen was collected in a 10-mL, evacuated, rubber-stoppered glass container to screen donors for HbsAg, HIV Ab, and HCV Ab. Donor units that tested positive for any of these three viruses were discarded and excluded from the pooling process. Within five to ten minutes of blood collection, the iced whole blood units were weighed and placed into a Beckman J6B refrigerated centrifuge equipped with a six-place rotor with carriers and spun at 2,200 rpm (1,500 x g) for eight minutes at 4 °C. Following centrifugation, plasma was aseptically transferred from the plastic collection bag into a sterile 250-mL glass bottle equipped with a Teflon®-lined screw-cap closure. The material was held at room temperature for three to four hours to permit clot formation.

Clotted samples were centrifuged at 3,500 rpm for 18.5 minutes (2,700 x g) using a Sorval RT6000 equipped with H1008 rotor and four single-slot carriers. Sera was transferred to 250-mL Wheaton borosilicate bottles capped with Teflon®-lined screw caps and stored at 4 °C.

The evacuated tube was collected from each donor immediately after the whole blood unit was used to analyze individual units for exclusion criteria. ELISA techniques using Abbott Laboratories reagents for HbsAg, HIV Ab, and HVC Ab screened each unit for positive reactivity. (See Section B5.)

Sixty 1.0-mL frozen aliquots were prepared from each serum unit selected for the pool preparation. A Wheaton Unispense set at 1.0 mL was used to prepare the aliquots in 3.0-mL glass vials, capped with Teflon®-covered gray stoppers and secured with center tear-out aluminum crimped seals. The aliquots from each individual unit were labeled and frozen at -70 °C. The residual material of the individual units was used for the pool preparation. The optimal serum volume from individual units was set at 120 mL; however, 60- or 90-mL volumes were used from units that met the selection criteria but had low yield. Therefore, 44 individual serum units were included in the low/normal serum pool, 37 of which provided 120 mL, 5 gave 90 mL, and 2 gave 60 mL for a total pool volume of 5,010 mL. Similarly, 43 individual serum units were used for the high-lipid pool with 29, 10, and 4 units providing 120, 90, and 150 mL each, for a total pool volume of 4,980 mL. Both pool preparations were completed within 42 hours of the initial whole blood collection. Each pool was incubated for 18 hours at 4 °C with constant mixing with a magnetic stirrer at low speed to ensure homogeneity of the final pool and allow any nonspecific agglutination or further clotting process to occur.

Following the cold incubation period, the serum pool was decanted into a 5-gal. (18.9-L) stainless steel tank. Nitrogen at low pressure (2 to 3 psi) was required for sterile filtration through a 0.22- μ m hydrophilic Durapore® Millipore filter into a 6.0-L glass flask prior to final aliquoting of the pool. As with the individual serum units, 1.0-mL aliquots of the pool were dispensed with the Unispenser into 3.0-mL

glass vials, capped, sealed, and frozen at -70 °C. The 6.0-L flask of pooled sera was maintained at 4 °C on ice and placed on a magnetic stirrer to ensure the homogeneity of the product throughout the aliquoting process. Boxes of the 1.0-mL vials of the pool were placed in a precooled -70 °C freezer. All of the sample boxes were placed in the freezer at the same time, being sure that each box was in direct contact with the metal shelf of the freezer. A total of 56 hours were required from the initial whole blood collection to completion of the aliquoting, capping, crimping, and storage of the pooled sera. The two pools were prepared within one week of each other and stored at -70 °C.

Key aspects of this approach for the preparation of two levels of lipid (cholesterol and triglyceride) are the following elements:

- Unaltered serum units (no additives).
- Fasting status of donors (minimize chylomicrons and large VLDL).
- Short time from collection to pool dispensing and freezing.
- Material maintained at 4 °C throughout the process.
- Glass vials as primary storage containers.
- Storage at -70 °C.

B5 Testing Procedures Relevant to Production of Cholesterol Pools

B5.1 Virus Testing

Individual donor units were tested for Human Immunodeficiency Virus, Hepatitis C Virus, and Hepatitis B Surface Antigen with Abbott EIA Diagnostic kits. The readings were made on an Abbott Quantum II spectrophotometer with internal controls provided with the Abbott System test kit.

B5.2 Microbial Testing

Microbial testing was performed with BBL media. The following plates were inoculated with a sample from each pool: nutrient agar, MacConkey agar, and blood agar. Additionally, a triple sugar iron (TSI) agar tube was inoculated with each pool. This combination of media was used to rule out the presence of *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. A *Staphylococcus* latex test was performed on colonies identified to be *Staphylococcus* to rule out *Staphylococcus aureus*.

B5.3 Analyte Testing

- (1) Total protein, bilirubin, A/G ratio, and glucose were tested on Hitachi 705 Automatic Chemistry Analyzer using reagents provided by the manufacturer, Boehringer Mannheim Corporation. Precical used for calibration and Precitrol (N, AbN) used as controls were manufactured by Boehringer Mannheim Corporation. The Hitachi 705 Chemistry Analyzer is maintained by the manufacturer twice a year in a preventive maintenance program. Daily controls are run for each analyte.
- (2) Cholesterol and triglycerides were tested on a Hitachi 705 Automatic Chemistry Analyzer using reagents provided by the manufacturer, Boehringer Mannheim Corporation. Standards and controls were frozen serum manufactured by Solomon Park Research Laboratories with target values set using the Abell-Kendall Reference Method at the Northwest Lipid Research Laboratories, University of Washington, Seattle and at the Centers for Disease Control and Prevention, Atlanta, respectively. The Hitachi 705 Chemistry Analyzer is maintained by the manufacturer twice a year in a preventive maintenance program.

Daily controls were run with cholesterol and triglyceride analyses. The Hitachi 705 was certified for cholesterol testing through the cholesterol Reference Method Laboratory Network at the Northwest

Lipid Research Laboratories, University of Washington, Seattle. Solomon Park also participates in the ReLabs program through the Northwest Lipid Research Laboratories, University of Washington, Seattle, which assesses accuracy and precision for total and HDL-cholesterol and triglycerides on a quarterly basis. Finally, Solomon Park participates in the CAP Excel proficiency testing program for lipids.

- (3) Ammonia was tested by end-point ultraviolet analysis on a Beckman DU-20 Spectrophotometer using reagents from Sigma Diagnostics. Calibration was conducted per Sigma's instructions. Controls were manufactured and provided by Sigma. The Beckman DU-20 Spectrophotometer is maintained by the manufacturer twice a year in a preventive maintenance program. Daily controls were run with each ammonia analysis.
- (4) Hemoglobin was tested by end-point colorimetric analysis on a Beckman DU-20 Spectrophotometer using reagents from Sigma Diagnostics. Standards and controls were manufactured and provided by Sigma. The Beckman DU-20 Spectrophotometer is maintained by the manufacturer twice a year in a preventive maintenance program. Daily controls were run with each hemoglobin analysis.
- (5) Clotting Element Test was tested by OD readings made before and after exposing the individual unit or pool specimen to 55 °C for five minutes. The readings were made on a Beckman DU-20 Spectrophotometer. The Beckman DU-20 Spectrophotometer is serviced by the manufacturer twice a year on a preventive maintenance contract.
- (6) pH was monitored on a Beckman f40 pH meter using a refillable combination electrode Ag/AgCl half cell. The meter was standardized at the beginning of each run with buffer solutions with target values at pH 4.00, 7.00, and 10.00.

B5.4 Blood Typing

Blood typing was performed by agglutination using Dade reagents and red-cell controls and was done using the "tube method."

B6 Materials Validation Results

A critical assumption that was made when combining donor units to produce serum pools was that the pool would behave similarly to individual patient specimens in the measurement process, and thus the pool could be used to establish traceability and assess measurement accuracy. To determine whether the pooling process contributed to matrix effects, the guideline was validated by preparing two serum pools according to the written specifications. The pools were evaluated for cholesterol measurement performance relative to the population of individual donor samples contributed to each pool. The results of this validation work is planned for archival publication and is therefore presented here in summary.

Two factors were considered when selecting the number of donors used to make each pool (other than serum volume):

- **The effect of an interference from one or more donors on the final pool.** The effect of an interference from one donor was estimated by assuming that bias was proportional to the donor's serum volume contribution to the pool. If this assumption was correct, the bias introduced into the pool was 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 cholesterol bias of 30% due to interferences, a 1%

bias in a pool comprised of 30 donors would result. Based on this assumption, a pool of at least 40 donors was selected to minimize the effect any single donor might have on the pool.

- **The sample size needed to estimate bias, with sufficient statistical power, in the final pool result.**
A statistical design was developed which permitted detection of performance differences in excess of 2% between the pool and the population of patient sera. Assuming that field methods for cholesterol measurement should perform with a CV of at least 3% (based on NCEP performance guidelines), it was determined that 40 donors are required to detect a 2% bias between the pool and donors with 90% confidence.

Based on these relationships, two pools were prepared from individual units as follows:

Pool 95-LI: 44 units of 60 to 120 mL each.

Pool 95-LII: 43 units of 90 to 150 mL each.

Twenty-six instrument/reagent systems representing 13 manufacturers and one reagent vendor participated in the materials validation study. Each participating manufacturer was provided with vials from the two pools and each of the donor units comprising each pool. Nineteen systems assayed all samples in a single analytical run with appropriate replication for the desired statistical power. The pools were tested 15 times, the donor units in duplicate, and two levels of controls in singlet at the beginning and end of the run. The remaining seven systems made two or more analytical runs. Results were coded so that the identities of instrument vendors were unknown.

None of the 26 instruments evaluated in this study showed a consistent bias between donor samples and serum pool cholesterol results. Bias is defined here as the difference between the observed serum pool mean and the expected mean calculated from the weighted average of the individual donor samples (bias = pool result - donor result). Pool result is the mean of all serum-pool assays. The donor result is the weighted mean of the donor sample assays. For systems with multiple runs, an additional weighting factor was computed from the controls in each run to minimize run effects. Weights were computed from the serum volumes for each donor used to create the pools. These weighted means are the "expected" values for the pools in the absence of matrix bias. All biases, with the exception of one system for Pool II, were less than the 2% criteria used to determine a statistical difference between the pools and the population of patient sera used to prepare the pools. In fact, all biases with the exception of one different system for Pool I and one different system for Pool II, were less than 1.5%. Except for the one system for Pool II, which at 2.2% was just slightly greater than the 2.0% criteria, the results in this study are sufficient to conclude that the serum pools prepared according to this guideline behave like donor samples on the systems evaluated.

B7 Certificates of Analysis for Cholesterol Pools

**SOLOMON PARK RESEARCH LABORATORIES
CERTIFICATE OF ANALYSIS FOR POOLS**

POOL ID # NCCLS I DATE/TIME OF POOLING 8:00 p.m./ 9/24/95

TECHNOLOGIST SM POOLING COMPLETED 11:00 p.m./ 9/24/95

TEST RESULTS:

AMMONIA:	[<u>76.6</u>]	MUST BE < 100 µmol/L
INSTRUMENT:	<u>BECKMAN DU-20</u>	METHODOLOGY: <u>UV, ENDP</u>
REAGENT:	<u>SIGMA</u>	LOT # <u>045H6189</u> EXP <u>3/96</u>
CALIBRATION:	<u>SIGMA</u>	LOT # <u>035H6119</u> EXP <u>3/97</u>

pH: [8.20] MUST BE 7.6 ± 0.4

INSTRUMENT:	<u>BECKMAN φ - 40</u>	METHODOLOGY : <u>ELECTRODE</u>
REAGENT:	<u>N/A</u>	LOT # <u>---</u> EXP <u>---</u>
CALIBRATION:	<u>VWR</u>	LOT # <u>268</u> EXP <u>5/96</u>

A/G RATIO:	[<u>1.37</u>]	MUST BE > 1.0
INSTRUMENT:	<u>HITACHI 705</u>	METHODOLOGY : <u>A/ (B-A)</u>
REAGENT:	<u>BMD</u>	LOT # <u>---</u> EXP <u>---</u>
CALIBRATION:	<u>PRECICAL</u>	LOT # <u>---</u> EXP <u>---</u>

(Calculated/NA)

GLUCOSE:	[<u>84.0</u>]	MUST BE > 60.0 mg/dL
INSTRUMENT:	<u>HITACHI 705</u>	METHODOLOGY : <u>HEXOKINAS</u>
REAGENT:	<u>BMD</u>	LOT # <u>14660</u> EXP <u>2/27/96</u>
CALIBRATION:	<u>PRECICAL</u>	LOT # <u>XLS-96</u> EXP <u>8/17/97</u>

MICROBIAL TESTING
NUTRIENT AGAR
OF COLONIES: 0 MUST BE < 10 CFU/mL
CFU/mL no growth

BLOOD AGAR
RESULTS: 0

Plates were run on final filtered pool as well as on unfiltered residue from pressure tank. There was no growth in either of these samples.

MACCONKEY AGAR
RESULTS: 0

TSI TUBE
RESULTS: 0

<i>Escherichia coli</i>	<u> </u> POS	<u> X </u> NEG
<i>Salmonella</i>	<u> </u> POS	<u> X </u> NEG
<i>Staphylococcus aureus</i>	<u> </u> POS	<u> X </u> NEG
<i>Pseudomonas aeruginosa</i>	<u> </u> POS	<u> X </u> NEG

B7 Certificates of Analysis for Cholesterol Pools (Continued)

CLOTTING ELEMENT TEST AND FINAL O.D. 710 nm READING

INITIAL O.D. 710 nm READING [0.051] *Pool 1*

POST 55° O.D. 710 nm READING [0.040]

FINAL UNIT O.D. 710 nm READING [0.040] MUST BE <0.5

F-ratio MS (B) /MS (A) [0.34] MUST BE <2.348

F-ratio MS (C) /MS (B) [1.59] MUST BE <3.020

POOL ACCEPTED YES NO IF NO CHECK HERE

FREEZING DATA:

DATE AND TIME OF INITIAL FREEZING OF POOL AND INDIVIDUAL DONOR UNITS ASSOCIATED WITH POOL 11:20 p.m. / 9/25/95

HAVE ANY VIALS BEEN EXPOSED TO TEMPERATURES OTHER THAN - 70 DEGREES CELSIUS FOLLOWING INITIAL FREEZING ? YES NO

IF YES, DESCRIBE TEMPERATURE AND DURATION OF EXPOSURE:

PRODUCTION/SHIPPING DATA:

DATE AND TIME PRODUCTION BEGAN (FIRST DONOR DRAW) 6:00 a.m. / 9/23/95

DATE AND TIME PRODUCTION ENDED (PRODUCTS FROZEN) 12:48 a.m. / 9/26/95

DATE AND TIME PRODUCT SHIPPED NA / NA

FORM COMPLETED BY: Patric A. Clapshaw Sci. Director
NAME TITLE

FORM VERIFIED BY: Sheila McGrath MLT (ASCP)
NAME TITLE

B7 Certificates of Analysis for Cholesterol Pools (Continued)

**SOLOMON PARK RESEARCH LABORATORIES
CERTIFICATE OF ANALYSIS FOR POOLS**

POOL ID # NCCLS II DATE/TIME OF POOLING 9:00 p.m./ 10/1/95
 TECHNOLOGIST SM POOLING COMPLETED 11:20 p.m./ 10/1/95

TEST RESULTS:

AMMONIA: [49.9] MUST BE < 100 μ mol/L
 INSTRUMENT: BECKMAN DU-20 METHODOLOGY: UV, ENDPT
 REAGENT: SIGMA LOT # 045H6189 EXP 3/96
 CALIBRATION: SIGMA LOT # 035H6119 EXP 3/97

pH: [8.18] MUST BE 7.6 \pm 0.4

INSTRUMENT: BECKMAN ϕ - 40 METHODOLOGY : ELECTRODE
 REAGENT: N/A LOT # --- EXP ---
 CALIBRATION: VWR LOT # 268 EXP 5/5/96

A/G RATIO: [1.28] MUST BE > 1.0
 INSTRUMENT: HITACHI 705 METHODOLOGY : A/ (B-A)
 REAGENT: BMD LOT # --- EXP ---
 CALIBRATION: PRECICAL LOT # --- EXP ---
 (Calculated/NA)

GLUCOSE: [86] MUST BE > 60.0 mg/dL
 INSTRUMENT: HITACHI 705 METHODOLOGY : HEXOKINAS
 REAGENT: BMD LOT # 14660 EXP 2/27/96
 CALIBRATION: PRECICAL LOT # XLS-96 EXP 8/17/97

MICROBIAL TESTING
 NUTRIENT AGAR MUST BE < 10 CFU/mL
 # OF COLONIES: 0 CFU/mL no growth

BLOOD AGAR
 RESULTS: 0

Plates were run on final filtered pool as well as on unfiltered residue from pressure tank. There was no growth in either of these samples.

MACCONKEY AGAR
 RESULTS: 0

TSI TUBE
 RESULTS: 0

<i>Escherichia coli</i>	<u> </u>	POS	<u> X </u>	NEG
<i>Salmonella</i>	<u> </u>	POS	<u> X </u>	NEG
<i>Staphylococcus aureus</i>	<u> </u>	POS	<u> X </u>	NEG
<i>Pseudomonas aeruginosa</i>	<u> </u>	POS	<u> X </u>	NEG

B7 Certificates of Analysis for Cholesterol Pools (Continued)

CLOTTING ELEMENT TEST AND FINAL O.D. 710 nm READING

INITIAL O.D. 710 nm READING [0.062] *Pool II*

POST 55° O.D. 710 nm READING [0.058]

FINAL UNIT O.D. 710 nm READING [0.058] MUST BE <0.5

F-ratio MS (B) /MS (A) [0.65] MUST BE <2.348

F-ratio MS (C) /MS (B) [1.58] MUST BE <3.020

POOL ACCEPTED X YES NO IF NO, CHECK HERE

FREEZING DATA:

DATE AND TIME OF INITIAL FREEZING OF POOL AND INDIVIDUAL DONOR UNITS ASSOCIATED WITH POOL 11:00 p.m. / 10/2/95
HAVE ANY VIALS BEEN EXPOSED TO TEMPERATURES OTHER THAN - 70 DEGREES CELSIUS FOLLOWING INITIAL FREEZING ? YES X NO

IF YES, DESCRIBE TEMPERATURE AND DURATION OF EXPOSURE:

PRODUCTION/SHIPPING DATA:

DATE AND TIME PRODUCTION BEGAN 6:00 a.m. / 9/30/95
(FIRST DONOR DRAW)

DATE AND TIME PRODUCTION ENDED 1:10 a.m. / 10/2/95
(PRODUCTS FROZEN)

DATE AND TIME PRODUCT SHIPPED NA / NA

FORM COMPLETED BY: Patric A. Clapshaw Sci. Director
NAME TITLE

FORM VERIFIED BY: Sheila McGrath MLT (ASCP)
NAME TITLE

Summary of Comments and Subcommittee Responses

C37-P, *Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Proposed Guideline.*

Abstract

1. Abstract (page i, line 4) suggestion to add: "...commutable for cholesterol measurement, with the purpose of assigning values to calibrators at the level of the manufacturer's measurement procedures and/or routine measurement procedures."
- **The subcommittee agrees with the comment in principle. The Abstract has been revised to provide additional clarification.**

Foreword

2. Foreword (page xiii, first paragraph, line 1): it is presumed to be known that elevated blood cholesterol is harmful to the human health condition. Suggestion to add: "...blood cholesterol, *purportedly a threat to the human health condition worldwide*, has been a primary objective...etc."
- **An introductory statement has been added to indicate the world health problem of elevated cholesterol and the risk for heart disease.**

Section 3

3. Page 1 refers to "Universal Precautions." At the recent NCCLS conference in Toronto I was interested to learn that it is the intention of the CDC to replace this term with "Standard Precautions." Based on several discussions, my understanding is that NCCLS would like to follow the CDC's lead and revise appropriate sections of guidelines and standards as they are developed or come up for review.
- **CDC has developed new guidelines (Standard Precautions) for use in the care of patients in acute-care hospitals. The new "Standard Precautions" synthesize 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. The subcommittee agrees that Section 3 should be retitled "Standard Precautions." The section has been revised accordingly.**

Section 4

4. The term: Reference Material(s): e.g., title, keywords, foreword etc. I submit to the committee: to use the term *calibrator* or *calibration material*. Often the term is also used for control material for the purpose of verification of trueness. To mix the two meanings creates confusion; I invite the committee to screen the text and see where which meaning is meant. I do think that Reference Material should not be used in the sense of "control material."
- **The use of the term is not ambiguous. The definition provided is derived directly from ISO vocabulary, as published in ISO Guide 30: 1992 and VIM: 1993, 6.13. This reference citation has been added in the text of the definition. ISO includes both controls and calibrators under the general heading of reference materials. A given material may be employed with a particular procedure for either one purpose or the other (calibration or control), but can never be used for both purposes within that particular procedure. The materials prepared in accord with this guideline may be used for lesser purposes, such as quality control, if desired, but the resulting quality of the materials allows for higher level use.**

5. The terms: Accuracy, trueness and precision: throughout the document, but particularly in the Foreword and Scope. All three terms are qualitative ones; e.g., high/low. The quantitative expression of trueness is percentage bias, and of precision, e.g., the percentage CV (imprecision). In this case the term "trueness" can be applied as the analyte which we want to measure – cholesterol – physico-chemically is fully defined, and the result of measurement can be expressed in terms of moles, i.e., SI units. I submit to the committee to screen the text for the occurrence of the term "accuracy," "accurate" where actually – in many cases – *trueness* or *true (value)* is meant. Please harmonize the terminology used in your document with the upcoming ISO/CEN documents on traceability.
- **This is an excellent suggestion and the subcommittee agrees with the comment. In order for NCCLS documents to be accepted globally, terminology should conform to ISO guidelines. However, accuracy, accurate are historically ingrained into the scientific vocabulary in the U.S. and are generally understood to mean the same as trueness, true. Trueness, true is the preferred ISO terminology; thus, the text has been changed accordingly. However, accuracy, accurate have been retained in parentheses throughout the document to maintain clarity of presentation. A discussion has been added in the definition section indicating that trueness is the ISO-recommended terminology. Appropriate changes are introduced in the Foreword and the Scope.**
6. The term: Commutability: this term is difficult to define. But, see the ISO/CEN documents on traceability for another – more elaborate – definition. Perhaps, the committee wants to incorporate that definition next to the one offered in the NCCLS document (perhaps a note?).
- **The ISO definition for commutability of a material has been added as a note to the definition of commutable in Section 4, Definitions.**
7. A hemolyzed specimen may show minimal color change. It should be noted that the color change is proportional to the extent of hemolysis.
- **The statement is correct but a revision in the definition of hemolysis is not required.**

Section 5.1

8. Pool preparation: 2nd paragraph: as this document might become universally applicable: a sentence about the legal issues of collection blood donations might be appreciated; it should be according to local legislation; the procedure should be transparent and informed consent from the donor should be obtained.
- **Section 6.1, Donor Selection, has been revised to indicate the need for informed consent from each donor.**
9. Pool preparation; 4th paragraph, line 10: sterile glass bottle: is the committee sure that "sterile" is actually meant, or will *aseptic* do as well? (Within CEN the debate on "sterile" and "aseptic" is quite heated). Cf. also page 10 of NCCLS document with the flowchart, and clause 7, (6), 7. (13).
- **In Section 7, the glass bottles, containers, and stir bars used in the blood processing steps (3), (6), and (12) were sterilized by autoclaving, and therefore the term sterile used in this context is appropriate. A sentence for clarification has been added to Section 5.1, Pool Preparation, 6th paragraph.**
10. Pool preparation; 5th paragraph, line 7: predominate = *predominant*?
- **The subcommittee believes that either term is acceptable.**

11. Pool preparation; 5th paragraph, line 11-12: "This peak was of minor consequence in all other samples." Is the committee sure that it was of minor consequence? How do you know? Potential interference.
- **It is known that lipoproteins undergo oxidation and degrade. It has also been shown that phthalate esters can promote lipoprotein oxidation. For convenience and practical reasons, collection of blood in plastic bags is the recommended industry standard. To minimize contact with the plastic bag, the collected blood was carefully transferred to glass as quickly as possible after the bags were centrifuged to separate the plasma from the cells. The two pools prepared in accordance with this guideline were compared with blood products collected and stored long-term in plastic bags. Compared to the stored blood bank product which gave a major phthalate ester peak by GC/MS, the two pools prepared in accordance with this guideline contained only trace amounts of detectable phthalates. Actual interference by the presence of any phthalate esters was not evaluated. It was assumed that, because the levels observed in the two study pools were so small, the impact on the materials was insignificant. The text has been revised to indicate that the potential for interference by the presence of phthalate esters was not investigated.**
12. Pool preparation; 11th paragraph: when making such materials – for other purposes and quite a long time ago - I was accustomed to snap-freeze the small volume aliquots (see B.1. aliquots of 1 mL). The mixture may be crude acetone/alcohol – dry ice mixture. The liquid is frozen within 15 minutes. Suggestion that the committee considers to recommend this procedure. Please note: if snap-freezing is mentioned, it is recommended to add a note to the effect that labels should be applied after this procedure is done (labels/felt-pen writing is affected by acetone/alcohol).
- **The subcommittee appreciates the comments and experience provided by the reviewer. The procedure detailed in this guideline only describes what was done and what worked. The subcommittee cannot speculate beyond this point as to what alternative approaches might be successful. The procedure described in the guideline is more practical for a manufacturing setting when preparing large pools. It would seem that the procedure using acetone/alcohol-dry ice mixture is more appropriate for freezing small pools prepared in a research setting.**
13. Pool preparation, 12th paragraph: suggestion to print the sentence "Material should not be refrozen" in bold print.
- **The sentence has been changed to bold print.**
14. Pool preparation; 3rd paragraph: From our experience an interference of a single donor is sometimes not linear after dilution with sera of other donors. Therefore, in this case you cannot minimize the bias in a linear way as proposed. In contrary, it may happen that the opposite will be observed.
- **It is certainly possible that some interferences may be nonlinear, and would not be minimized by dilution. The subcommittee selected this admittedly simplistic model merely to provide a means to statistically estimate the minimum number of donors required for the pools. Protein modification or precipitation may occur due to cross-contamination with one or more donor units. The cold incubation period of 18 hours should handle this; however, the unpredictability of interferences underscores the importance of performing commutability verification studies on each pool, as part of the routine quality assurance process, prerequisite to releasing a given pool for use as a reference material. This point has been added to the text in Section 5.2, Validation of Performance and Commutability.**

Section 5.2

15. Validation of performance and commutability: 3rd paragraph, line 2: "..... panel of individual human serum samples." Fresh, frozen, old samples?

- Although a full panel of fresh human sera is the ideal, it may not be practical to obtain a broad range of specimens covering the reportable range of the method/procedure in a time frame that would support such a study. A statement has been added to the text in Section 5.2 which discusses the practical implications of this problem. When specimens need to be preserved to support these studies, careful storage of the specimens at 70 ° Celsius is required.

Section 6.1

16. Donor selection: "...concentration levels ...": how many levels are desired? Should the levels cover the measuring range claimed by the manufacturer? This will be required in the ISO/CEN standard on traceability.

- The guideline outlines procedures for selecting, processing, and combining donor units to prepare frozen serum pools for cholesterol measurement. Specification of concentration levels of resulting pools prepared according to the guideline is outside the context and purpose of this document. The two concentration levels were selected to have a normal cholesterol level pool and an elevated cholesterol level pool based on the NCEP cholesterol medical decision points for coronary heart disease risk. However, the guideline should be applicable to the preparation of cholesterol pools at all concentration levels. The number of pools and levels prepared would be based on the needs of the end user. A practical limitation to this process is the collection of sufficient donor units with values at the extremes of clinical interest to prepare sufficiently large pools.

The proposed requirement in the ISO draft standard on traceability (ISO/CD 17511; 1998, 4.3.2) covers the situation where a set of human samples is used as a secondary calibrator to ensure commutability of a reference material. In this case the set of samples should span the measuring interval of the method.

Section 7

17. (14): If possible, in the laboratory, we would like to recommend to freeze the pool with liquid air (shock-frozen pool). If the pool is frozen slowly, lipid particles are built. In dry chemistry these particles may be filtered by the separation layer with the result of a bias.

- See response to Comment 14.

Appendix B

18. B.2.3. Does the committee want to make a statement, please, about which reference measurement procedures are recommended to assign values for cholesterol and triglycerides?

- The Laboratory Standardization Panel of the NCEP recommends that all total, HDL, and LDL cholesterol and triglyceride results be standardized and traceable to the reference methods at the CDC. Therefore, if assigned, reference values should be established by the CDC reference methods.

Reference values were not needed as part of this project. The only values needed were the cholesterol values determined by each respective instrument system being evaluated for matrix effects from pooling.

19. B.3.2. Reagents: reagents for syphilis testing are not mentioned (see 6.2 (1)).

- The donor units were not tested for syphilis. Reference to syphilis testing in Section 6.2 has been deleted.

20. B.4. Blood collection and processing; 1st paragraph, line 4. Instead of "specimens" it is suggested to use "donations." The term "specimen" is regarded as ambiguous.

- **The term specimens is replaced with donor units.**

In addition, the term patient sample has been replaced with human sample throughout the document.

21. B.5.3. and B.7. : it seems to me that a "control material," called Precical, and other control materials by Sigma (for ammonia) and VWR (for pH) were used to calibrate the reagents and instruments. Control materials should – in my opinion – not be used for calibration. Perhaps I am mistaken; however, could this be clarified by the committee?

- **Different calibrator and controls were used for total protein, bilirubin, A/G ratio, and glucose. Specifically, Precical from Boehringer Mannheim Corporation (BMC) was used for calibration for total protein, bilirubin, A/G ratio, and glucose; and Precitrol (N, AbN) from BMC was used as controls. The text has been revised to indicate the use of Precitrol as a control. Separate and different calibrator and controls were provided by Sigma for the determination of ammonia.**

22. B.6, last paragraph: It is concluded that the serum pools tested behaved like donor samples on the 26-instrument/reagent systems. This appears to contradict the "exception" statement on lines 8 and 9.

- **The summary paragraph was revised to clarify the conclusions.**

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- 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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