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Guidance for 510(k)s on Cholesterol Tests for Clinical Laboratory, Physicians' Office Laboratory and Home Use

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Guidance for 510(k)s on Cholesterol Tests for Clinical Laboratory, Physicians' Office Laboratory and Home Use

This is a flexible document presenting current guidance on the preparation of premarket notifications (510(k)s) for cholesterol in vitro diagnostic devices employing enzymatic methodologies. It is based on 1) current basic science, 2) clinical experience, 3) previous submissions by manufacturers to the Food and Drug Administration (FDA), and 4) the Safe Medical Devices Act of 1990 and regulations in the Code of Federal Regulations (CFR). So that we may revise the draft as necessary, please send your comments to the address given below.

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PURPOSE:

This document is an adjunct to the CFR and FDA 87-4224, The In Vitro Diagnostic Devices: Guidance for the Preparation of 510(k) Submissions manual. It is not to supersede those publications, but provides additional guidance and clarification on what information is necessary before the FDA can clear a device for marketing.

DEFINITION OF DEVICE:

This generic type of device is intended for use in clinical laboratories and physicians' office laboratories (POLs) and home use as an in vitro diagnostic test for quantitative or qualitative measurement of cholesterol by enzymatic methodology.

PRODUCT CODE(S): CHH

REGULATION NUMBER: 21 CFR § 862.1175

- (a) Identification. A cholesterol (total) test system is a device intended to measure cholesterol in whole blood, plasma and serum. Cholesterol measurements are used in the diagnosis and treatment of disorders involving excess or low cholesterol in the blood and lipid and lipoprotein metabolism disorders.

(b) Classification. I

PANEL: Clinical Chemistry 75

REVIEW REQUIRED: 510(k)

I. BACKGROUND

Elevated serum cholesterol (hypercholesterolemia) is one of the major risk factors associated with an increased risk of coronary heart disease (CHD)¹. To help identify and treat individuals at risk for CHD because of hypercholesterolemia, the National Heart, Lung, and Blood Institute (NHLBI) initiated a National Cholesterol Education Program (NCEP) and established the Laboratory Standardization Panel (LSP) on Blood Cholesterol Measurement. The LSP's study and report on the current state of reliability of cholesterol measurements in clinical laboratories² and the recommendations it made toward improved cholesterol measurements³ are the basis for the primary issues to be addressed in this document.

The major laboratory goals of the NCEP are: achieving the level of precision and accuracy recommended by the Panel for quantitative cholesterol assays and the standardization of cholesterol measurements which ensures accuracy that is traceable to the National Reference System for Cholesterol (NRS/CHOL). The NRS/CHOL includes the National Institutes of Standards and Technology (NIST) definitive method and the CDC reference method^{4,5} together with the NIST Certified Reference materials. This program was designed to implement a national standardization program that will allow laboratories and manufacturers to trace cholesterol measurements back to the reference system.

The colorimetric procedures that employ strong acid containing reagents (e.g., Liebermann-Burchard) have now been replaced by enzymatic procedures in the clinical laboratory⁶. The Liebermann-Burchard reagent used in the CDC reference method is based on the method of Abell, et.al.,⁴. The colorimetric procedures were generally replaced by the enzymatic methods in the clinical laboratories because the use of enzymes improves specificity without pretreatment and includes reagents that are less corrosive.

In the early to mid-1980s compact analytical systems emerged. These compact systems are portable, designed for ease of use and require minimal user intervention. This allowed rapid laboratory testing in the physician's office and off-site environments. These systems are capable of measuring whole blood without prior separation of red cells utilizing solid phase separators or

filtrators that separate plasma from whole blood.

Non-instrumented technologies began to emerge in late 1980s and early 1990s. These systems use enzymatic strip technology, allowing the qualitative or quantitative measurement of cholesterol. The principle of the chemical action that occurs is the same as that employed by the compact analyzers.

II. DEVICE DESCRIPTION

Cholesterol, is transported in the blood in combination with specific proteins in complexes known as lipoproteins. These are classified as chylomicrons, high density, low density, very low density and intermediate density lipoproteins (HDL, LDL, VLDL, and IDL respectively), and differ in terms of both protein and relative lipid composition. Total cholesterol values reflect the total amount of cholesterol in the lipoproteins, and are determined by chemical or enzymatic methods. The specimen used may be whole blood, serum or plasma using either heparin or EDTA as anticoagulant (cholesterol measurements should not be determined from fluoride, citrate, or oxalate treated specimens).

Currently the majority of cholesterol test systems available utilize enzymatic methodology based on the use of coupled enzymatic reagent systems and various chromagens. The methodology is applicable to both manual and automated procedures, including compact desk-top analyzers and non-instrumented systems used in physicians' offices. Some of these systems have the capability of accepting whole blood samples (venous or fingerstick) and automatically removing the cells⁷.

A. Principle of the Test

The most common enzymatic method employs the Trinder reaction which includes the breakdown of cholesterol esters to free cholesterol by cholesterol esterase. Cholesterol oxidase, in the presence of oxygen, oxidizes free cholesterol to form cholest-4-ene-3-one and hydrogen peroxide. The hydrogen peroxide reacts with phenol and 4-aminoantipyrine in the presence of peroxidase (POD) to form a quinoneimine dye. In the most popular methods, phenol or other chromophores may be substituted. Enzymatic procedures may involve a catalase reaction sequence or amperometric measurement of either the oxygen consumed or that of hydrogen peroxide generation. The resulting color is measured by reflectance photometry. The non-instrumented systems allow for visual determination of cholesterol by use of chromogens or indicator systems.

B. Merits and Limitations of Various Test Methodologies

Enzymatic methods are more specific, permitting direct measurements without a preliminary extraction or other pretreatment. Reagents used in the enzymatic methods are less corrosive and can be used in manual systems as well as the complex automated instrumentation. With all enzymatic procedures, deterioration or variation in the source of the enzyme can occur. These effects can manifest as lot-to-lot and instrument-to-instrument imprecision⁸. Good Manufacturing Practices and laboratory quality assurance programs which include proficiency testing adequately address these concerns.

C. Specimen Type(s)

The specimen used may be whole blood, serum or plasma using either heparin or EDTA as anticoagulant (cholesterol measurements should not be determined from fluoride, citrate, or oxalate-treated specimens). Since the LSP guidelines reflect serum cholesterol determinations, it is recommended that EDTA plasma values should be converted to serum values according to the following factor: Serum cholesterol = Plasma cholesterol x 1.03, because plasma levels may be approximately 3% lower than serum values³. Additionally, please note that systems which utilize whole blood (fingerstick or venous) specimens actually measure plasma after a separation step. Because of the biases for cholesterol measured in fingerstick as compared to venous specimens, comparative studies between the two are indicated^{7,9}.

III. SPECIFIC PERFORMANCE CHARACTERISTICS

A. Overview

FDA requests different types and amounts of data and statistical analyses in applications to market in vitro diagnostic devices. The amount and type of data requested depends on: 1. the test analyte, 2. the intended use (which determines whether the application is a 510(k), an original Premarket Approval Application (PMA), or a supplement to a PMA), 3. whether the test is quantitative or qualitative, and 4. whether the type of data design is independent or paired. Additional data may be necessary to substantiate certain claims of intended use or clinical significance. Test performance characteristics may be based on just one specimen type if data of sufficient sample sizes presented in the submission adequately demonstrates there is no statistically significant difference (with reasonable statistical power) in test results between different sample types. FDA recommends that performance characteristics be

obtained with all sample types that demonstrate statistical differences. Use appropriate statistical studies, e.g., regression studies or paired-T test for paired data, or student's independent T-test for normally distributed data or other appropriate non-parametric tests, to support the data.

B. Analytical/Laboratory/In Vitro Studies

1. Goals

- a) Show that the performance of the device is substantially equivalent to another legally marketed device and comparable to the Abell-Kendall reference method.
- b) Provide data determined with the device to support performance parameters specific to and important for operating the device, e.g., reproducibility.
- c) Justify the use of the test with all claimed specimen types.

2. Performance Characteristics

- a) Include the following performance characteristics as appropriate:
 - 1) Analytical Sensitivity (Detection Limit) and Linear Range

Determine the analytical sensitivity (detection limit) and/or the linear range of the assay using 40 normal and 40 abnormal specimens, including samples with low, mid-range and high levels of cholesterol¹⁰.
 - 2) Specificity/Cross-Reactivity/Interference Studies
 - a) Test as appropriate for the following possible cross reactive or interfering substances using the assay system:
 - i) Proteins, hormones, etc., found in the specimen under normal or diseased conditions that possess similar chemical structure or epitopes as the test analyte.
 - ii) Sample integrity conditions, e.g., lipemia, hemolysis, bilirubinemia, heat inactivation, freeze-thawing, etc.
 - b) Alternatively, add a statement to the Limitations

section of the package insert that the device has not been tested for cross-reactivity or interference of one or more substances.

- c) Test any potentially cross-reacting endogenous substances at extremely high concentrations using the assay system. Testing should include common serum components, such as lipids, hemoglobin, bilirubin, and other endogenous substances that may interfere^{11,12,13}.
- d) Test for any potentially cross-reacting exogenous substances^{11,12,13}. Exogenous materials such as commonly used or commonly co-administered drugs that might interfere with the determination also should be tested for interferences.
- e) Interference studies should be performed to assess the effects of other substances or conditions on the cholesterol, e.g., heat inactivation, freeze-thawing, etc..

3. Precision studies^{11,12}

- a) The National Committee for Clinical Laboratory Standards (NCCLS) recommends¹⁴ an analysis of variance experiment (ANOVA). In 510(k)s for all cholesterol test systems, FDA recommends that this or a comparable precision study protocol be followed, in addition to the precision studies required for the Certificate of Traceability.

It is optimal to conduct the ANOVA with controls or patient specimens at or very near the medical decision limits 200 and 240 mg/dL. These should be performed in duplicate for at least five days on at least two levels¹⁴. More runs and days may increase confidence in the results. Precision may be calculated as between-run and within-run percent coefficients of variation (%CV) and standard deviations (SD). However, an acceptable alternative is to calculate within-run and total %CVs and SDs. For all quantitative tests, these data should be kept on file by the manufacturer but not included in the 510(k). How to present the precision results in the package insert is discussed under IV. Labeling Considerations.

- b) For use in physicians office laboratories (POL), on-site precision evaluation at three independent sites should be performed. At each of the three sites, the

precision of the device should be evaluated as described in a).

- c) The reproducibility of qualitative tests is best demonstrated in studies of 1) the random error of visual interpretation by individual observers interpreting duplicate tests on a single sample source and 2) the random error of multiple observers interpreting a single test. Additional kinds of precision studies may be useful in demonstrating substantial equivalence. Precision studies for some qualitative devices may be unique to the device's design/format. We recommend discussing these unique studies with FDA early in the device development process.

4. Stability

According to Good Manufacturing Practices (GMPs), the manufacturer must maintain a file on the stability of all of the components of the device. The manufacturer does not have to submit this data to the FDA, but must be able to provide the data if it is requested to establish the safety and effectiveness of the device.

5. Specimen Collection and Handling

State specimen storage conditions in the package insert that is based on data on file or appropriate literature references in the submission. In unique test systems or truly novel specimen collection/handling, studies may be necessary to substantiate such claims.

Biological variability can be reduced by drawing blood under standardized conditions as recommended by NCEP³. The specimen may be whole blood, serum or plasma using either heparin or EDTA as anticoagulant. See section II.C. concerning plasma samples. Additionally, NCEP recommends that cholesterol measurements should not be made from plasma derived from fluoride, citrate or oxalate treated specimens.

C. Clinical Data

1. Comparison Studies

The performance of the device should be compared with the Abell-Kendall reference method performed in a CDC-certified Cholesterol

Reference Method Network Laboratory, CRMLN. These comparison studies may be conducted with samples split between the manufacturing site or clinical laboratory site and the CRMLN. In the 510(k), the manufacturer should submit a copy of the Certificate of Traceability obtained from the CRMLN. The CRMLN can provide you with a complete detailed protocol for the study required to obtain this certification.

When submitting the Certificate of Traceability from the CRMLN, studies on the accuracy of the device compared with a legally marketed predicate device need not be conducted. Only a descriptive comparison of the technologies and features should be provided. We recommend presenting this information in tabular form in the 510(k).

The LSP has recommended that bias (systematic deviation from the true value) of current cholesterol measurements should not exceed 3 percent CV from true value³. FDA has adopted these recommendations and advises that all devices subject to 510(k) clearance perform within these guidelines or be labeled that they do not.

The Abell-Kendall method is performed with serum only. Traceability of other sample types (alternative matrices such as fingersticks, plasma, whole blood, etc.) should be compared to serum on the device in question and the serum values compared to the reference method. Because of the potential for bias between cholesterol measured in fingerstick compared to venous specimens, comparative studies between the two sample types should be included for the device in question when fingerstick sample is claimed for use.

2. Study Design

a) Most cholesterol test systems currently marketed are quantitative, a qualitative cholesterol test system may demonstrated to be substantially equivalent. The data analysis differs for these two kinds of tests. Our recommendations for each are given below. In all cases, qualitative and quantitative, and all intended use settings, in order to ensure that observations are distributed independently and randomly, each patient should be sampled only once.

1) Quantitative tests

Compare results obtained using all types of samples claimed in the submission. Samples from 40 patients, free from interfering substances should be used. Submit these

samples to the CRMLN for assay with the Abell-Kendall reference method. You are not required to compare results from the currently, legally marketed predicate device with the reference method. Please contact the CRMLN for detailed instructions on submitting specimens to them. The study design that demonstrates substantial equivalence depends on the intended use setting, please see e) (next page) for additional guidance.

2) Qualitative tests

Data are required to demonstrate traceability of qualitative cholesterol device to the Abell-Kendall reference method. In this case, the study design depends on the intended use setting, see the following discussion in section b). Its substantial equivalence to a legally marketed predicate device is determined by its performance compared with the Abell-Kendall reference method.

However, the data analysis for qualitative devices is the same for any intended use setting. We recommend that data be presented in the form of 3 x 3 tables for each NCEP category as well as in tabular form comparing the currently legally marketed predicate and CRMLN results. These data should also be presented as 2 x 2 tables, one table with the borderline and high groups collapsed into one group to examine the device's clinical specificity and one table with the normal and borderline groups collapsed to examine the device's clinical sensitivity. The confidence intervals for the sensitivity and specificity should be presented. Provide the mean and range of Abell-Kendall results for each NCEP classification group, Normal, Borderline and High as assigned by the device.

- b) The study design required to demonstrate substantial equivalence of a cholesterol test system depends on the intended use of the test system. Our recommendations about study designs are based on whether the test will be used in clinical laboratories, physician's offices or over the counter. Each is discussed below.

1) Clinical Laboratory Use

For quantitative devices intended for use in clinical laboratories, each venous sample should be split into enough aliquots to perform comparisons to the predicate device as well as with the reference method. The tests using the device and the predicate may be conducted at the manufacturing site, however, one aliquot should be sent to

a CRMLN for analysis by the reference method. A copy of the Certificate of Traceability should be submitted in the 510(k).

2) Physicians' Office Laboratory Use

For POL use devices which are quantitative and intended for use with venous specimens, aliquots of venous samples should be split between the POL site and the manufacturing site for testing. The study should consist of a minimum of 3 POL sites with 40 clinical samples at each POL site.

The manufacturing site should have a Certificate of Traceability for the device tested at the manufacturing site or a clinical laboratory. Aliquots split between the POL site and the CRMLN are not required for quantitative POL devices that use only venous specimens.

However, POL use devices that are qualitative, and/or factory calibrated and/or self contained or intended only for use with fresh fingerstick samples should be studied by direct comparison with the CRMLN. In these studies, a venous specimen for testing at the CRMLN should be obtained from each subject immediately after the fingerstick. The study should consist of a minimum of 3 POL sites with 40 subjects at each POL site. We recommend using 3 lots of product in these studies.

3) Over the Counter Use

A laboratory evaluation of the analytical performance of the device (e.g., sensitivity, specificity, accuracy and reproducibility) should be done. The purpose of this evaluation is to establish the performance characteristics of device as determined under controlled conditions. In addition, the device should be extensively studied in consumer field evaluations as described in Appendix I.

IV. LABELING CONSIDERATIONS

The presence and concentrations of various endogenous substances such as vitamin C, bilirubin or other reducing compounds, hemoglobin from hemolysis or large lipoprotein particles may influence cholesterol measurements. The presence of protein, certain salts, detergents or stabilizing agents can influence some enzymatic methods. Instability, chemical, or biological contamination, and improper storage conditions can affect the precision and accuracy. All of these concerns are important to effective use of the test and should be addressed in the labeling.

Assure that the labeling complies with § 502(a) and § 502(f)(1) of the Act that the directions for use are not false or misleading and that directions for use are adequate. 21 CFR § 801.119 states that all in vitro diagnostic devices shall be deemed to be in compliance with the requirements of 502(a) and 502(f)(1) of the act if they meet the requirements of 21 CFR § 809.10, labeling for in vitro diagnostic products. The emphasis of this document is to explain some of the points in the above publications. In determining whether a device is misbranded because the labeling or advertising is misleading, § 201(n) of the Act permits the following to be taken into account among other things:

"(n) If an article is alleged to be misbranded because the labeling or advertising is misleading, then in determining whether the labeling or advertising is misleading there shall be taken into account (among other things not only representations made or suggested by statement, word, design, device, or any combination thereof, but also the extent to which the labeling or advertising fails to reveal facts material in the light of such representations or material about the consequences that may result from the use of the article to which the labeling or advertising relates under the conditions of use prescribed in the labeling or advertising thereof or under such conditions of use as are customary or usual."

All abbreviations and acronyms should be defined. In addition to the basic requirements of 21 CFR § 809.10, the following information should be carefully reviewed.

1. The Intended Use Statement

Labeling should provide a concise description of the essential information about the product. A typical intended use statement is: ABC's cholesterol test is an enzyme assay for the quantitative (or qualitative) determination of cholesterol in whole blood for use in physicians' office laboratories to screen for elevated cholesterol as a risk factor in coronary artery disease. The intended use statement should include the following information:

- 1) Whether the assay is quantitative or qualitative
- 2) Analyte
- 3) Test methodology
- 4) Whether the assay is to be used only with a specific

instrument

- 5) Specimen type(s)
- 6) Whether it is for use in clinical laboratories, doctors' offices, or home use (The Limitations section should include any specific training required for test performance or use.)
- 7) Whether it is for screening, or to aid in the diagnosis as an adjunct to other procedures
- 8) Clinical significance, if it can be stated in a few words. (If the clinical significance is lengthy or complicated create a separate heading entitled "Clinical Significance".)

2. Conditions for Use

A statement concerning the test results for qualitative devices should be added to the package insert. A typical statement should include the following information.

"Qualitative tests provide preliminary analytical results. All results indicating elevated blood cholesterol levels should be verified by a quantitative cholesterol method. Clinical considerations and professional judgment should be applied to the interpretation of results by this test."

3. Specimen Collection and Handling

Biological variability can be reduced by drawing blood under standardized conditions as recommended by NCEP³. The specimen may be whole blood, serum or plasma using either heparin or EDTA as anticoagulant. See section II.C. concerning plasma samples. Additionally, NCEP recommends that cholesterol measurements should not be made from plasma derived from fluoride, citrate or oxalate treated specimens.

Provide the following information:

- a) The type of specimen to be collected, e.g., whole blood, plasma. Fingertick samples should provide a free-flowing drop of blood. Excessive squeezing or milking should be avoided.
- b) The patient should sit quietly for about 5 minutes before the sample is drawn.

- c) References for appropriate collection procedures, e.g., NCEP, NCCLS guidelines, textbooks, journals, etc.
- d) The amount of specimen required, both optimum and minimum.
- e) Interfering substances or conditions.
- f) The specimen storage instructions and stability periods.

4. Directions for Use

Instructions should be adequate for the intended site and user of the device.

5. Calibration and Quality Control

The difference between calibration materials and quality control materials should be clearly distinguished. The cholesterol levels of calibration materials must be known with a greater degree of certainty than those of control materials.

a) Calibrators

The values of calibrators used should be assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL). Calibration materials should have concentrations around the decision levels. For total cholesterol appropriate calibrator levels range from 200 to 300 mg/dL. A statement that the calibration materials have assigned values traceable to the NRS/CHOL should be included in the package insert.

Because of the possible discrepancies from matrix effects with some calibrator materials, the manufacturer should acknowledge that the processing and additives to these materials may affect cholesterol measurement characteristics in certain devices.

b) Quality Control Materials

The LSP recommends two levels of controls, one in the normal range (175-200 mg/dL), and one near the concentrations for intervention (240-260 mg/dL). Statements that the control materials are intended for use only as monitors of accuracy and precision and that the values listed are provided only as guides should be included in the package insert.

6. Interpretation of Results/Expected Values

To ensure uniformity in interpretation of cholesterol results the cholesterol cutpoints for identifying adults with high or moderate cholesterol levels (issued by the NCEP Adult Treatment Panel)^{2,3} should be included in the labeling.

The labeling should refer to the following NCEP classifications:

Below 200 mg/dL (<5.17 mmol/L) Desirable blood cholesterol
200-239 mg/dL (5.17-6.18 mmol/L) Borderline-high blood cholesterol
240 mg/dL and above (≥6.21 mmol/L) High blood cholesterol

Labeling should include statements that at least two measurements of cholesterol on separate occasions should be made before any medical decision is made since a single point total cholesterol measurement may not represent a patient's usual cholesterol concentration, and Cholesterol results around the decision points should be followed with a repeat measurement.

7. Limitations of the Test

A statement should be made that the preanalytical factors can affect cholesterol measurements.

It should be indicated that there may be a tendency of portable cholesterol analyzers to underestimate values because of fingerstick sampling. During fingerstick sampling, excessive squeezing or milking may dilute the sample and produce erroneous results.

For POL tests, include a limitation stating that an elevated cholesterol result should be confirmed by a follow-up testing in a clinical laboratory. FDA considers this necessary given that the NCEP recommended goals for bias and imprecision³, both 3% CV, suggest that for a single analysis, the allowable total error would be +8.9%. Considering that the cutpoints for borderline and high cholesterol differ by only 40 mg/dL and adding the biological variability of 5-6%, there is an appreciable variation.

If the test is claimed for use with EDTA plasma, add a statement such as "It is recommended that if EDTA plasma is used, the plasma value should be converted to a serum value according to the following factor: Serum cholesterol = Plasma cholesterol x 1.03 because plasma levels may be

approximately 3% lower than serum values."³

Include a limitation statement that the test does not meet the NCEP recommended goals for bias and imprecision, if that is observed in the studies conducted for the 510(k).

8. Performance Characteristics

a) Comparison

The labeling should summarize the information on the Certificate of Traceability, and if conducted, the results of any additional comparison studies deemed necessary by FDA. These would studies of the device used at 3 POL sites or results from consumer field evaluations of home use tests. In these cases, present the number of samples tested, the range of cholesterol concentrations, the correlation coefficient, the means for both methods, bias and the regression equation. For qualitative devices, account for all values in the indeterminate or equivocal range.

b) Precision

The labeling should summarize the results of the recommended precision studies. For all quantitative tests, summarize the results as the mean, standard deviation and coefficient of variation for each level used in within-run and run-to-run or within-run and total precision studies. Describe the number of assays performed, and whether control materials or pooled patient samples were used. For qualitative tests, report the random error of visual interpretation by individual observers interpreting duplicate tests on a single sample source and the random error of multiple observers interpreting a single test or results observed in comparable studies.

c) Sensitivity

The lowest detectable concentration, or the typical absorbance change corresponding to the lowest detectable cholesterol concentration under the conditions of the assay may be stated as an indication of sensitivity.

d) Assay range

The range of assay linearity should be stated in the package insert.

e) Interfering substances

Interferences or lack thereof should be indicated in the package insert. Possible interferences may be ascorbic acid, bilirubin, hemoglobin, and lipemia. For effects of various drugs, Young et. al.¹⁷ should be referenced.

9. Professional Use Products

For professional use products the following legend is required by 21 CFR 801.109. "Caution: Federal law restricts this device to sale by or on the order of a _____", the blank to be filled with the word "physician" or descriptive designation of any other practitioner licensed by the law of the State in which he/she practices to use or order the use of the device.

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Appendix I Points to Consider for Home Use Cholesterol Tests:

Division of Clinical Laboratory Devices' policy for home use cholesterol suggests the study design shown in Table 1. Manufacturers should conduct consumer field evaluations in which the subject's test is compared with the professional's test with both of these results compared directly to the CRMLN. Presumably any OTC device will require a fingerstick sample, therefore a venous sample must be obtained from each subject for testing at the CRMLN. For quantitative devices, the correlation coefficient obtained from regression analysis should be no less than 0.90. Also conduct analytical studies of the device as described in section B. Analytical/Laboratory/In Vitro Studies.

TABLE 1

SUBJECT PERFORMED/SUBJECT READ
compared with
PROFESSIONAL PERFORMED/PROFESSIONAL READ

SUBJECT PERFORMED/SUBJECT READ
compared with
SUBJECT PERFORMED/PROFESSIONAL READ

SUBJECT PERFORMED/SUBJECT READ
compared with
CRMLN

PROFESSIONAL PERFORMED/PROFESSIONAL READ
compared with
CRMLN

Perform consumer field evaluation studies as outlined in the Home-Use In Vitro Diagnostics Guidance Document¹⁵ and NCCLS Document GP14, Labeling of Home-Use In Vitro Testing Products¹⁶.

Study design should include three geographically distinct sites, with at least 400 subjects and with adequate protocol control to allow pooling. The sample size is important for calculating confidence intervals for sensitivity and specificity. The sample size should be determined prior to the initiation of the study. There is no unique statistical answer. The number depends upon the precision (width of the confidence intervals) being claimed for those proportions (sensitivity, specificity).

Subjects should be observed without coaching. The number of fingerstick attempts required to obtain an adequate sample should be documented and provided in the 510(k). The subjects must not

receive any prior training in the use of the device, nor be allowed to practice the use of the device beforehand. The evaluation should mimic actual use conditions as closely as possible. The consumer sample should represent a mix of intended users with varying ages, occupations and educational levels. Ethnic diversity should reflect that of the general population. Please include all demographic data in the 510(k).

Present results in tabular form from the consumer field evaluation described above showing the number of subjects in each National Cholesterol Education Program classification, i.e., desirable, borderline and high. Show the number of subjects misclassified by the home use result compared to Abell-Kendall result. The manufacturer should include the misclassification rate as an adjunct to the accuracy statement given in the labeling.

A large study (>400 subjects) is suggested in order to obtain a population of normally distributed results within the range of the test. Please provide complete subject accountability, i.e., disposition of every subject (and the corresponding cholesterol result) enrolled in the study.

Provide the following parameters based on the analysis of the consumer field evaluation described above, sensitivity, specificity, accuracy, positive predictive value and negative predictive value. Include the 95 percent confidence interval for each performance parameter. The study should be designed such that defensibly small confidence intervals result. In the case of qualitative tests, the analysis recommended earlier in this document for prescription use devices should be followed.

Submit a thorough risk/benefit discussion of the intended use (home use) of the device. Expert witness opinion in writing may be requested in support of significant new claims or differences from predicate devices, including device design or format issues.

Provide data to show that various technique anomalies, expected with untrained users, such as touching the finger to the sample well or cell separator, wide variations in sample volume and dropping the sample over only a partial area of the cell separator will not affect the result or place the subject at risk of obtaining a false result.

Evaluate the effects of sample volume and hematocrit ranges on the accuracy of the device, taking into account that untrained users may not have experience with microliter volumes and most will not know whether their hematocrits are within normal ranges.

Include an evaluation of the labeling for grade level and comprehension. Labeling should meet the guidance previously published^{15,16} and address concerns specific to home use cholesterol. These include exclusion of hemophiliacs and patients on anti-coagulant therapy, discussion of CHD risk factors, and instructions for obtaining an adequate blood specimen.

The manufacturer should state in the 510(k), the regulatory status of any ancillary medical device, such as lancets that will be included with the marketed device.

Appendix II Quantitative Cholesterol Test Checklist

Use this checklist only for quantitative cholesterol tests intended for use in clinical and/or physicians' office laboratories. Please use this checklist to ensure the completeness of your 510(k) submission.

For cholesterol tests that are qualitative, factory calibrated, single use or intended only for use with fresh fingerstick samples, please use Appendix III.

- CDRH Premarket Submission Cover Sheet (suggested).
- Truthful and Accurate statement verbatim as required by 21 CFR 807.87(j). Additions and deletions are not permitted.
- 510(k) summary **or** statement as required by 21 CFR 807.92 or 21 CFR 807.93 respectively.
- Certificate of Traceability obtained from a CDC-certified Cholesterol Reference Method Network Laboratory, CRMLN.
- Precision results in the labeling (do not submit data) at or near the medical decision 200 and 240 mg/dL.
- Labels and labeling as required by 21 CFR 809.10 (b).
- Labeling limitation **if** the test does not meet current National Cholesterol Education Program (NCEP) guidelines for bias and/or precision.

For POL tests (quantitative tests using only venous specimens):

- Data from 3 POL sites with 40 clinical samples at each site. (Direct comparison of the POLs and CRMLN are not required for quantitative POL devices that use only venous specimens.)
- Precision results at or near the medical decision 200 and 240 mg/dL, from studies conducted at the 3 POL sites.
- Information on the nature and location of each POL.
- Information on the education and training of all individuals at each performing the assay at each POL.

Appendix III Qualitative Cholesterol Test Checklist*

Use this checklist for submissions for qualitative cholesterol tests. Please use this checklist to ensure the completeness of your submission.

*Also use this checklist for quantitative POL tests that are factory calibrated, single use or use fresh fingerstick samples.

- CDRH Premarket Submission Cover Sheet (suggested).
- Truthful and Accurate statement verbatim as required by 21 CFR 807.87(j). Additions and deletions are not permitted.
- 510(k) summary **or** statement as required by 21 CFR 807.92 or 21 CFR 807.93 respectively.
- Labels and labeling (package insert) as required by 21 CFR 809.10 (b).
- Reproducibility at or near the medical decision levels 200 and 240 mg/dL, from the 3 POL sites.
- Bias at the medical decision levels 200 and 240 mg/dL, from the comparison with Abell-Kendall (quantitative fingerstick, single use or factory calibrated).
- Limitation in the labeling **if** the test does not meet current National Cholesterol Education Program (NCEP) guidelines for bias and/or precision.

All POL cholesterol tests:

- Information on the nature and location of each POL.
- Information on the education and training of all individuals at each performing the assay at each POL.

Single use/factory calibrated POL tests using venous specimens:

- Data from 3 POL sites with 40 clinical samples at each site. (Direct comparison of the POLs and CRMLN are not required for quantitative POL devices that use only venous specimens.)

Appendix III Qualitative Cholesterol Test Checklist page 2

All POL cholesterol tests that use fingerstick specimens:

- Data from 3 POL sites with 40 clinical samples at each site compared with the CRMLN. For qualitative, single use or factory calibrated tests that use fingerstick specimens, a venous specimen is obtained for shipment to the CRMLN.

All Qualitative Tests:

- Demonstration of the random error of visual interpretation by individual observers interpreting duplicate tests on a single sample source.
- Demonstration of the random error of multiple observers interpreting a single test.
- Comparison data in 3 x 3 tables for each NCEP category as well as in tabular form comparing the new device with the currently, legally marketed predicate and CRMLN results. Provide the mean and range of Abell-Kendall results for each NCEP classification group, Normal, Borderline and High as assigned by the device.
- Comparison data in 2 x 2 tables, one table with the borderline and high groups collapsed to examine the device's clinical specificity and one table with the normal and borderline groups collapsed to examine the device's clinical sensitivity. Provide the confidence intervals for the clinical sensitivity and specificity.
- Reproducibility at or near the medical decision levels 200 and 240 mg/dL, from precision studies conducted at the 3 POL sites.

Appendix IV Home Use Cholesterol Test Checklist

Use this checklist for submissions for home use cholesterol tests. Please use this checklist to ensure the completeness of your submission.

- CDRH Premarket Submission Cover Sheet (suggested).
- Truthful and Accurate statement verbatim as required by 21 CFR 807.87(j). Additions and deletions are not permitted.
- 510(k) summary **or** statement as required by 21 CFR 807.92 or 21 CFR 807.93 respectively.
- Labels and labeling appropriate for home use, evaluated for reading level and comprehension. Discuss in the labeling repeat and/or professional testing for results near medical decision points, and emphasize that changes in diet or medication should never be based on the results of any home test.
- Labeling showing the number of subjects and percent misclassified (i.e., false negative and positive rates) by the home use test compared to Abell-Kendall result.
- Precision data at the medical decision levels 200 and 240 mg/dL for quantitative tests.
- Bias at the medical decision levels 200 and 240 mg/dL, from the comparison with Abell-Kendall for quantitative tests.
- Interfering/cross reacting substance data.
- Limitation in the labeling **if** the test does not meet current National Cholesterol Education Program (NCEP) guidelines for bias and/or precision.
- Evaluation of various technique anomalies, expected with untrained users, touching the finger to sample well or cell separator, wide variations in sample volume, dropping the sample over only a partial area of the cell separator and any other anomalies unique to the format of the device.
- Data from consumer field evaluations at three geographically distinct sites, with at least 400 subjects. Appropriate samples should be drawn from these subjects for testing at CRMLN. Also collect device results obtained by untrained subjects without assistance and device results obtained by healthcare professionals from these subjects in a masked

study.

Appendix IV Home Use Cholesterol Test Checklist page 2

- Each of the following data analyses, including linear regression correlation coefficients > 0.90 :
 - [SUBJECT PERFORMED/READ] vs [PROFESSIONAL PERFORMED/READ]
 - [SUBJECT PERFORMED/READ] vs [SUBJECT PERFORMED/PROFESSIONAL READ]
 - [SUBJECT PERFORMED/READ] vs CRMLN venous result
 - [PROFESSIONAL PERFORMED/READ] vs CRMLN venous result
- Table showing the number and percent of subjects performing 1, 2, 3, and more than 3 fingerstick attempts in order to obtain an adequate sample.
- Subject demographic data showing a representative mix of age, ethnicity, occupation and educational level.
- Comparison data in 3 x 3 tables for each NCEP category as well as in tabular form comparing the new device with the currently, legally marketed predicate and CRMLN results.
- Demonstration that data are normally distributed results along the concentration range covered by the method.
- Complete subject accountability, i.e., disposition of every subject enrolled and the corresponding cholesterol result.
- A thorough risk/benefit discussion of the device including significant new claims or differences from predicate devices, and unique device design or format issues.
- The regulatory status of any ancillary medical device, such as lancets that will be included with the marketed device.

Appendix IV Home Use Cholesterol Test Checklist page 3

All Qualitative Home Use Tests:

- Demonstration of the random error of visual interpretation by individual observers interpreting duplicate tests on a single sample source.
- Demonstration of the random error of multiple observers interpreting a single test.
- Comparison data in 3 x 3 tables for each NCEP category as well as in tabular form comparing the new device results with the CRMLN results. Provide the mean and range of Abell-Kendall results for each NCEP classification group, Normal, Borderline and High as assigned by the device.
- Comparison data in 2 x 2 tables, one table with the borderline and high groups collapsed to examine the device's clinical specificity and one table with the normal and borderline groups collapsed to examine the device's clinical sensitivity. Provide the confidence intervals for the clinical sensitivity and specificity.
- Reproducibility at the medical decision levels 200 and 240 mg/dL, from precision studies conducted at the 3 POL sites.