PTS Diagnostics
CardioChek® Plus Comparison Study

Evaluation Protocol:
Accuracy
Precision

CardioChek Plus Lipid+eGLU
Smart Bundle

For Use in Comparisons to a
Reference Laboratory

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Recommended Evaluation Protocol:

1. Scope

This protocol provides direction for a comparison study of the CardioChek Plus analyzer to a reference laboratory.

2. Overview of Studies and Expected Results

2.1. Accuracy Study:

This is a comparison study using venous samples from a single venipuncture and one fingerstick sample from each subject. A minimum of 20 subjects will be evaluated.

The fingerstick sample shall be evaluated on the CardioChek Plus analyzer only. The serum from the venous sample red top serum tube will be split into two aliquots. One serum aliquot will be tested by the laboratory evaluating the system using the reference analyzer of their choice, and the other aliquot will be packaged and sent to a reference laboratory or PTS Diagnostics by overnight courier to be tested. Results from either the reference laboratory or PTS laboratory will be used as the method comparison reference.
A lithium heparin (green top) whole blood tube will also be collected from the same venipuncture site for precision and glucose spikes (optional).

IMPORTANT NOTE: Fingerstick and venous blood samples must be collected at the same time. The serum from the venous sample must be separated within 30 minutes of the blood draw. Fingerstick and venous blood collected at different times cannot be compared.

a. Evaluation by Average Difference

The difference between the CardioChek Plus result and the laboratory result is calculated as (CardioChek Plus result -Reference result). The differences are calculated and expressed as a percentage of the reference result. The differences are then averaged.

The **average difference** is expected to be equal to or less than:

Total cholesterol:	±10%
HDL cholesterol:	±12%
Triglycerides:	±15%
Glucose:	ISO 15197 2013 standard: 95% of results in the following categories: <100 mg/dL: ±15 mg/dL and/or ≥100 mg/dL: ±15%

NOTE: This value is the average difference of the population; differences between individual results are expected to vary both above (+) and below (-) the average difference value.

b. Linear Regression

Linear regression is the generally accepted statistical approach to analyzing paired comparison data and describing the relationship of one method to another. In a regression model, the key performance measures are the slope of the regression equation line, the y-intercept (*i.e.*, that point at which the regression line crosses the y axis of the graph) and the correlation coefficient (r), which represents the degree of variability for points around the regression line. Optimally, the slope should be close to 1.0 and the intercept near 0.0. This is often not observed due to the limited range of the sample results and the small sample number tested. Regression analysis is only appropriate when results cover the entire measuring range of the test.

The correlation coefficient expressed as (r) should be greater than 0.88.

The results of a linear regression may also be used to estimate the predicted result of the tested method (e.g., CardioChek Plus analyzers) based on the result of the reference method (e.g., laboratory analyzer). Typically, this is done at clinical decision limits, e.g., for total cholesterol: 160, 200, and 240 mg/dL.

2.2. Precision Study:

Precision is defined as the ability of a test system to reproduce a given result for a single test sample. Optimally, this is determined using at least ten replicates of a single whole blood sample. The number of replicates (n), mean (average), standard deviation (SD), and percent coefficient of variation (%CV) are calculated for the replicates (n), The %CV is an estimate of the precision of the system. The %CV of the whole blood replicates on the CardioChek Plus analyzer is expected to be <10% for total cholesterol, HDL cholesterol, triglycerides and glucose.

3. Protocol Overview and Execution Planning

3.1. Study Location:

The site coordinator will establish a suitable, temperature-controlled setting for conducting the evaluation. The setting should be one in which the study subjects can be comfortably seated while being tested. Provisions will be made to collect the venous and fingerstick blood under aseptic conditions using standard venipuncture and fingerstick methods. The temperature of the testing environment should be between 20-27°C and the humidity less than 80%. Test operators will be those individuals familiar with the operation of the CardioChek Plus analyzer.

The protocol requires the testing of test strips supplied by the PTS Diagnostics study coordinator. Each test result will be associated with a subject number, site, date, and an operator name.

3.2. Personnel/Training:

For this evaluation it is preferable to use trained operators and/or PTS Diagnostics Technical Support Specialists. As a CLIA-waived product, the CardioChek Plus analyzers have been demonstrated to produce acceptable results when used by operators with no previous experience with the system. If the site is interested in an additional operator evaluation, this can be performed as a second evaluation. Such operators should be given the instructions for use (IFU) to review prior to conducting the study to ensure that users follow recommended operational procedures and techniques.

It is very important that the CardioChek Plus operator has read all instructions for use provided with the PTS Diagnostics products including the analyzer and test strips. PTS Diagnostics Customer Service is available toll-free at 877-870-5610 to answer questions regarding the CardioChek Plus system.

Improper technique in sample collection, storage, and handling of test strips or general use of the products may affect both accuracy and precision of results.

3.3. System Setup:

a. Insert fresh batteries in the analyzer.
b. Ensure the optical window (glass) is clean. Re-clean if necessary, as indicated in the user guide.
c. Run the gray Check Strip to verify the optics and electronics of the CardioChek Plus analyzer are functioning properly.
d. Run the PTS Diagnostics liquid controls to verify the CardioChek Plus system is functioning properly.
e. Log Check Strip result, control results, equipment GLP information, temperature, humidity, and capillary tube lot information on the Evaluation Information Form (Appendix A).

3.4. Subject Selection:

A minimum of 20, with an optimal of 40, subjects should be evaluated so that the data is statistically relevant. The ideal assay ranges for the subjects selected should encompass the dynamic range of the test strips and be distributed to the extent possible as indicated in the table below. The n (number of samples) indicated in the table assumes optimal enrollment of 40 subjects total. Note that it is often difficult to fill the higher range. In these instances where the desired number of subjects cannot be obtained in any bracket, additional subjects should be added to the mid-range to fulfill the total number of subjects desired. The more subjects that can be used the greater the confidence in the analysis of the comparison; thus, 20 subjects are the minimum and 40 subjects are preferred.

Sample Distribution Table

TEST	MEASURING RANGE	RANGE % (n) Samples	RANGE % (n) Samples	RANGE % (n) Samples	RANGE % (n) Samples	RANGE % (n) Samples
Total Cholesterol	100-400 mg/dL	100-160 mg/dL 15% (6)	161-199 mg/dL 25% (10)	200-239 mg/dL 25% (10)	240-280 mg/dL 25% (10)	>280 mg/dL 10% (4)
HDL Cholesterol	20-120 mg/dL	20-35 mg/dL 15% (6)	36-45 mg/dL 25% (10)	46-55 mg/dL 25% (10)	56-70 mg/dL 25% (10)	>70 mg/dL 10% (4)
Triglycerides	50-500 mg/dL	50-100 mg/dL 15% (6)	101-150 mg/dL 25% (10)	151-200 mg/dL 25% (10)	201-300 mg/dL 25% (10)	>300 mg/dL 10% (4)
Glucose	40-600 mg/dL	40-100 mg/dL 15% (6)	101-175 mg/dL 25% (10)	176-225 mg/dL 25% (10)	226-400 mg/dL 25% (10)	>400 mg/dL 10% (4)

3.5. Sample Collection and Handling:

This study requires subjects to be fasting for a minimum of 9 hours**. It is important to note on the result log if a patient presents and is drawn as a non-fasting subject.** Always collect fresh whole blood using accepted phlebotomy technique avoiding excessive blood cell trauma causing lysing of the cells. It may not be possible to observe cell lysis (hemolysis) in the whole blood specimen.

Should hemolysis be observed, the sample must be eliminated from the study analysis.

a. CardioChek Plus Testing

i. Capillary Sample: Samples must be tested immediately upon collection.
ii. Venous Samples: Fresh anticoagulated whole blood should be tested within one hour.

b. Laboratory Testing Serum Sample

i. Serum

Most laboratories prefer to run lipid and glucose analysis using a serum specimen. Thus, this sample must be collected in a tube without anticoagulant (red top). This sample is allowed to clot, and then is centrifuged, aliquoted, and sent to the desired laboratory.

ii. Plasma

Alternatively, if a heparinized specimen is acceptable in the laboratory, a second lithium heparin blood collection tube (green top) should be collected, centrifuged, and the plasma sent to the laboratory for analysis of total cholesterol, HDL cholesterol, triglycerides and glucose.

IMPORTANT: The CardioChek Plus analyzer is a whole blood analyzer. Serum and plasma are not appropriate samples for the CardioChek Plus analyzer accuracy/precision study.

4. Evaluation Procedure

4.1. Accuracy by method comparison:

a. Turn the CardioChek Plus analyzer on. Insert the MEMo® Chip for the lot specific test strips being used. Insert an eGLU test strip, then a Lipid Panel test strip into the analyzer.
b. The display should read APPLY SAMPLE. If the CardioChek Plus analyzer displays RUN TEST, press the Enter button to access the INSERT STRIP and APPLY SAMPLE screens.
c. Perform the fingerstick. Apply a blood sample to the tip of the eGLU test strip. Wipe away any remaining blood with gauze and collect a sample with a 40 µL capillary collection device.
d. When the CardioChek Plus analyzer displays blood drop icons over the test strip on the left of the display screen, dispense the blood sample onto the blood application window of the Lipid Panel test strip. (The CardioChek Plus analyzer will automatically begin testing the sample.)
e. When the CardioChek Plus analyzer displays the results (CHOL, HDL, TRIG and eGLU), record them on the Raw Data Collection form in Appendix B. (Note if the subject is non-fasting.)
f. Turn the used Lipid Panel test strip over and confirm that all three reaction circles on the back of the test strip are completely and evenly colored. If not, retest with an unused test strip.
g. Clean and disinfect the analyzer according to the manufacturer's recommendations.
h. To test the next sample, press the Enter button until the display reads INSERT STRIP. Insert eGLU test strip, then a Lipid Panel test strip. Repeat steps (b) through (g).

4.2. Preparation of glucose spikes (if applicable):

a. Thaw the Glucose Spiking Solution aliquot prior to use.
b. The concentration is approximately 20 g/100 mL (20,000 mg/dL).
NOTE: Final concentration of the spiked sample is measured after the glucose spike is added to each whole blood sample.
c. If the glucose concentration of the sample is less than desired, spike the sample(s) with Glucose Spiking Solution. Use the following equation to estimate the volume of the spiking solution to add to the blood sample(s):

$$[(C_T - C_C)V_C] / 20 = V_S$$

C_S = Concentration Glucose Spike (mg/dL) approximately 20 g/dL (20,000 mg/dL)

C_C = Current Glucose sample concentration (mg/dL) or "have"

C_T = Target sample Glucose concentration (mg/dL) or "want"

V_C = Sample blood volume (mL)

V_S = Spiking Glucose solution volume (µL)

d. Prepare only one (1) to three (3) "spikes" at a time.
e. Allow the sample to equilibrate for at least thirty (30) minutes at room temperature.
f. Invert the "spike" gently but thoroughly and analyze the sample on the CardioChek Plus analyzer. Record the results on the Raw Data Collection form in Appendix B.
g. Centrifuge the remainder of the "spike."
h. Prepare the samples for reference analyzer analysis in the same manner as the subject samples.

4.3. Precision Study (Same Day)**:**

a. Select three (3) venous subject samples from those collected for the correlation study.
   i. Samples should be selected such that they display CardioChek Plus results near the three analyte ranges for each analyte listed in the table below. Lithium heparin (green top) tubes selected for precision studies should remain capped until testing begins.

Analytes	Ranges in mg/dL		
	Level 1	**Level 2**	**Level 3**
Cholesterol	130-160	180-210	220-250
HDL Cholesterol	30-40	45-55	60-75
Triglycerides	90-120	140-180	200-250
Glucose	60-90	120-180	220-280

a. <u>eGLU Only (n=10)</u>:

- Gently invert the tube 4-5 times.
- Test each sample 10 times on the CardioChek Plus analyzer with an eGLU test strip.
- Apply sample with either a precision pipette or transfer pipette.
- When the heart stops beating, hold down the NEXT button (on right) until the result appears on the screen.
- Record the results on the Precision Form in Appendix C.
  NOTE: All glucose testing must be performed as quickly as possible as glucose in whole blood decreases at a rate of about 5-10% per hour.

b. <u>Lipid Panel Only (n=10)</u>:

- Gently invert the tube 4-5 times.
- Hold down the NEXT button (on right) until the eGLU strip icon is replaced with a strikeout icon. ⃠
- Insert the Lipid Panel strip only.
- Apply sample with a 40 µL capillary collection device.
- Results will display “eGLU ---" on the screen.
- Test each sample 10 times on the CardioChek Plus analyzer (Lipid Panel only), gently inverting the tube between each run.
- Turn the used test strips over and confirm that the three reaction circles on the back side of the test strips are completely and evenly colored. If not, retest with a fresh unused test strip. Note in the comments section of the data form if there was insufficient sample placed on the strip or that the strip was unevenly colored. This result should be excluded from the analysis.
- Record the results on the Precision Form in Appendix C.

5. Data Analysis

NOTE: All data submitted to PTS Diagnostics Technical Group shall be analyzed as described below. A report will be issued after completion and review of the data analysis.

5.1. Accuracy- Two Statistical tools for analyzing accuracy will be used: Average Difference and Correlation by linear regression:

a. Average Difference

i. When the laboratory results are received, complete the lab results column on the data collection form.

ii. For each subject sample, calculate the difference and % difference between the CardioChek Plus result and the lab result using the following formulas

- CardioChek result – Reference Laboratory result = Difference (Bias)
- (Difference / Laboratory result) x 100 = % Difference

NOTE: If the CardioChek Plus result is greater than the laboratory result, the difference will be a positive (+) number. If the CardioChek Plus result is less than the laboratory result, the difference will be a negative (-) number. Results that are outside the reportable range of the analyzer (report as < or >) and results that are clearly an error should be excluded from the analysis but should be noted in the comments and explained to the best of the operator's ability.

iii. Average Difference Calculation. Determine the mean (average) percent difference of all the sample results by adding the percent differences for each sample and dividing by the number of samples.

iv. Interpretation. The CardioChek Plus test system is performing acceptably if the mean difference for all the results is within previously established parameters.

b. Linear Regression:

This data is presented graphically with a descriptive linear regression equation, for example:

CardioChek Plus Result = (Slope) (Ref Lab result) + y-intercept

Predicted results can be calculated at the clinical decision limits and a table created to assist in managing expectations of end users. The table displays the predicted CardioChek Plus result and the percent difference between the reference laboratory result and the predicted CardioChek Plus result. For the Lipid Panel test strip analytes, the clinical decision limits evaluated are:

- Total cholesterol: 160, 200, 240, and 280 mg/dL
- HDL cholesterol: 40, 60, 80, and 100 mg/dL
- Triglycerides: 100, 150, 200, and 250 mg/dL
- Glucose: 100, 150, 200, and 250 mg/dL

These tables then allow the prediction of an average bias between systems across the clinically significant range.

5.2. Precision Study:

a. For the ten (10) replicates of each subject sample, calculate the mean (average), standard deviation (SD), and percent coefficient of variation (%CV).

b. The CardioChek Plus test system is performing acceptably if the coefficient of variation (%CV) of total cholesterol, HDL cholesterol, triglycerides, and glucose from this precision study are <10%.

6. Conclusion and Recommendation

The collective analyses of the accuracy and precision data are used to assess the CardioChek Plus test system and provide assurance that the system is giving the expected results. Each lot of test strips are calibrated, and the calibration is stored in the MEMo Chip which provides results that are calibrated to reference analyzers results; this is done during the routine manufacturer testing of the test strip lot. Precision reflects the variability across different test strips as they are tested on the CardioChek Plus analyzer. The selection of the reference laboratory and the laboratory analyzer in use can significantly influence the observed results. While all laboratory analyzers are typically capable of producing accurate and reproducible results, it has been established in proficiency testing studies and in the published literature that a variance exists across analyzers with respect to reported total cholesterol, HDL cholesterol, triglycerides, and glucose results. It is thus important to interpret all laboratory results with this known variability in mind. Once the data has been analyzed as described, conclusions for the results will be reported and comments and appropriate recommendations will appear in the final report.

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Appendix A: Control Results

Testing Environment

Temperature:

Humidity:

PTS Collect Capillary Tubes

Lot #:

Expiration Date:

Equipment GLP #

Pipettes:

Centrifuge:

Temp/Humidity Logger:

Check Strip Passed (Yes/No):

PTS Panel Test Strip Lot #

Expiration Date:

Liquid Controls

Multi Chemistry Control Lot #

Expiration Date:

HDL Control Lot # (if applicable)

Expiration Date:

PTS Panel Test strip Lot #

Expiration Date:

Date	CardioChek Plus Serial Number (Software Version)	Level 1				Level 2				Pass/Fai
		Chol	HDL	Trig	eGLU	Chol	HDL	Trig	eGLU	

Control Range Information

Analyte(s)	Level - 1	Level - 2
Cholesterol		
HDL		
Triglycerides		
Glucose/eGLU		

Appendix B: Raw Accuracy Data Collection Form

(All results are in mg/dL)

Evaluation Site: **Operator:**

Lot #: **Date:**

Expiration Date: **Page_____of _____**

Sample ID	Fasting/Non-Fasting (F/NF)	CardioChek Plus SN				Comment
		Cholesterol	HDL	Trig	eGlu	

Appendix C: Precision Form

Evaluation Site: **Operator:**

Lot# **Date:**

Expiration Date:

CardioChek Plus Analyzer SN				
Sample ID				
Analyte (mg/dL)	Cholesterol	HDL	Triglyceride	eGLU
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

CardioChek Plus Analyzer SN				
Sample ID				
Analyte (mg/dL)	Cholesterol	HDL	Triglyceride	eGLU
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

CardioChek Plus Analyzer SN				
Sample ID				
Analyte (mg/dL)	Cholesterol	HDL	Triglyceride	eGLU
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				