



**PTS Diagnostics
CardioChek[®] Plus Comparison Study**

**Evaluation Protocol:
Accuracy
Precision
Clinical Correlation
CardioChek Plus Lipid + eGLU[®]
Smart Bundle[™] Pack**

**For Use in Comparisons to a
Reference Laboratory**

Recommended Evaluation Protocol:

1. Scope

This protocol provides direction for a comparative study of the CardioChek[®] Plus analyzer to a reference laboratory.

2. Overview of Studies and Expected Results

2.1. Accuracy Study:

This is a comparative 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 venous sample red top serum tube will be split into two aliquots. One aliquot will be tested by the laboratory doing the evaluation on the reference analyzer of their choice and the other aliquot will be packaged and sent to an alternate facility by overnight courier to be tested. PTS Diagnostics has a qualified laboratory facility that can optionally serve as the alternate site. A lithium heparin tube (green top) will also be collected from the same venipuncture site for precision.

NOTE: Failure to use the blood collected at a single time point in this study will void the results as the lipid results from separately collected whole blood specimens can vary considerably. This is due to blood acquisition techniques and different times (and days) of collection.

a. Evaluation by Average Difference

The difference between the CardioChek Plus result and the laboratory result is calculated in a pair-wise fashion. The average of the differences is calculated.

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

Total cholesterol:	±10%
HDL cholesterol:	±12%
Triglycerides:	±15%
Glucose:	ISO 15197 2003 standard: 95% of results in the following categories
	<75 mg/dL: ±15 mg/dL and/or ≥75 mg/dL: ±20%

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

b. Linear Regression

Linear regression is the generally accepted statistical approach to analyzing paired 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 linear regression may also be used to estimate the predicted result of a new method (e.g., CardioChek Plus analyzers) based on the result of the standard 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 ten replicates of a single whole blood sample. The number of replicates 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 sequential subject number 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 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 completed PTS Diagnostics product training. It is also important to read all instructions for use provided with the PTS Diagnostics products. 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 PTS Diagnostics liquid controls to verify the CardioChek Plus system is functioning properly.

3.4. Subject Selection:

A minimum of 20, with an optimal 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 is a minimum and 40 subjects is 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	15-100 mg/dL	15-35 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 an aseptic technique, and avoid excessive blood cell trauma causing lysing of the cells. It may not be possible to observe cell lysis 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 analysis using a serum specimen. Thus, this sample must be collected in a tube without anticoagulant. This sample is allowed to clot, and then is centrifuged, aliquoted, and sent to the desired laboratory.

ii. Plasma

Alternatively, if a heparin 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. Correlation Study For Each Subject:

- 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 remaining blood with gauze and collect a sample with a 40µL 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 on the data collection form. (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. To test the next sample, press the Enter button until the display reads INSERT STRIP. Insert eGLU strip, then a lipid panel strip. Repeat steps (b) through (g).

4.2. 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 low, mid, and high analyte range of linearity for each analyte. Lithium heparin (green top) tubes selected for precision studies should remain capped until testing begins.

Analytes	Ranges in mg/dL		
	Low Range	Mid Range	High Range
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. eGLU Only (n=10):
 - o Gently invert the tube 4-5 times.
 - o Test each sample 10 times on the CardioChek Plus analyzer with an eGLU test strip.
 - o Apply sample with either a precision pipette or transfer pipette.
 - o When the heart stops beating, hold down the NEXT button (on right) until the result appears on the screen.
 - o Record the results.
- b. Lipid Panel Only:
 - o Gently invert the tube 4-5 times.
 - o Hold down the NEXT button (on right) until the eGLU strip icon is replaced with a strikeout icon. Ⓢ
 - o Insert the lipid panel strip only.
 - o Apply sample with a 40µL capillary collection device.
 - o Results will display "eGLU ---" on the screen.
 - o Test each sample 10 times on the CardioChek Plus analyzer (lipid panel only), gently inverting the tube between each run.
 - o 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.
 - o Record the results.

For each sample, calculate n, the mean, SD and %CV and record results on the data collection form provided.

5. Data Analysis

NOTE: All data submitted to PTS Diagnostics Technical Group shall be analyzed as described below. A formal report will be issued to the site for their records.

5.1. Average Difference:

- a. When the laboratory results are received, complete the lab results column on the data collection form.
- b. For each subject sample, calculate the difference and % difference between the CardioChek Plus result and the lab result using the following formulas
 - i. CardioChek result – Reference Laboratory result = Difference (Bias)
 - ii. (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.

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

5.2. Linear Regression:

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

$$\text{CardioChek Plus Result} = (\text{Slope})(\text{Ref Lab result}) + \text{Intercept}$$

This can then be applied to 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 strips 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.

5.3. 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. Data Interpretation, Sources of Error, and Final Recommendation

The collective analyses of the correlation and precision data are used to assess the CardioChek Plus test system and provide assurance that the system is giving the expected results. Bias is typically controlled in the CardioChek Plus analyzer by means of the MEMo Chip which provides a lot specific conversion of the CardioChek Plus results to reference laboratory results; this is done during the routine manufacturer quality control 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 comparator laboratory and the laboratory analyzer in use can significantly influence the observed differences. While all laboratory analyzers are typically capable of reproducing results in a precise manner, 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.



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