Polymer Technology Systems, Inc.
CardioChek® PA Comparison Study

Evaluation Protocol:
Accuracy
Precision
Clinical Correlation
PTS Panels® Lipid Panel Test Strips

For Use in Comparisons to a Reference Laboratory

Recommended Evaluation Protocol:

1. Scope

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

2. Overview of Studies and Expected Results

2.1. Accuracy Study:

This is a comparative study using two venous samples from a single venipuncture and a single fingerstick sample from each subject.

One venous sample will be evaluated on the CardioChek PA analyzer and the other transported to the laboratory for analysis. The fingerstick sample shall be evaluated on the CardioChek PA analyzer only.

NOTE: Failure to use the same blood specimen 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 PA 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:

Total cholesterol:	±10%
HDL cholesterol:	±12%
Triglycerides:	±15%

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.

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 (*i.e.,* CardioChek PA) based on the result of the standard method (*i.e.,* Laboratory). 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 (10) replicates of a single whole blood sample. The mean (average), standard deviation (SD), and coefficient of variation (CV %) is calculated for the replicates. The CV % is an estimate of the precision of the system. The CV % of 10 whole blood replicates on the CardioChek PA analyzer is expected to be <10% for total cholesterol, HDL, and triglycerides.

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. Two venous samples from the same venipuncture will be collected. There will also be a single fingerstick specimen collected and tested on an additional CardioChek® PA analyzer. The temperature of the testing environment should be between 20-27°C and the humidity less than 80%. The room temperature will be recorded before, during, and after testing. Test operators will be those individuals familiar with the operation of the CardioChek PA analyzer. All data will be recorded on the data collection sheets and later transferred to a Microsoft Excel file.

The protocol requires the testing of PTS Panels® test strips supplied by the PTS, Inc. 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. As a CLIA-waived system, the CardioChek PA 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.

Before testing, it is very important for the CardioChek PA operator to read all instructions, especially the IFU that comes with the test strips and the IFU that is packaged with the CardioChek PA analyzer. PTS, Inc. Customer Service is available toll-free at 877-870-5610 to answer questions regarding the CardioChek PA test 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:

- Insert fresh batteries in the analyzer.
- Ensure the optical window (glass) is clean. Re-clean if necessary as indicated in the user guide.
- Use the CardioChek ChekMate™ quality control kit to verify that the analyzer's electronics and optics are functioning properly at multiple levels. This is simply done by inserting the ChekMate MEMo Chip® in the analyzer and following the prompts on the screen and inserting the ChekMate strips, Level 1 and Level 2, sequentially. The results are displayed and must be compared to the acceptable ranges. Record the results. This must be done every day there is a subject test conducted.
- Run the provided liquid controls (Level 1 and Level 2) to verify the analyzer, test strip, and MEMo Chip are functioning properly together. The controls will be provided by PTS, Inc. Results should be recorded on the data collection sheet. The collection sheet should indicate which meter and lot number of strips were used for the QC test. It is only necessary to test one Level 1 and one Level 2 per lot of test strips.
- Liquid controls should be repeated at study completion.

3.4. Subject Selection:

A minimum of 20 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 PTS Panels® test strips assays 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)

3.5. Sample Collection and Handling:

Always collect fresh whole blood using an aseptic technique, and avoid excessive blood cell trauma causing lysing of the cells. It will not be possible to observe cell lysis in the whole blood specimen.

Should hemolysis be observed in the centrifuged blood, which is sent to the laboratory for testing, that subject must be eliminated from the study analysis.

a. CardioChek® PA Testing:
   i. Venous Sample
      For this comparison study, a lithium heparin (green top tube) anticoagulated specimen will be obtained. Mix the venous samples well by gently inverting the collection tube 7-8 times after collection (to avoid hemolysis). Test the venous samples within one hour after collection.
   ii. Capillary Sample
      The CardioChek PA analyzer may be used with either fresh or anticoagulated whole blood. During evaluations, it is strongly recommended that fingerstick samples be collected with a lithium heparin coated capillary pipette to allow additional time in the sample handling. Fingerstick samples must still be tested immediately.

b. Laboratory Testing Serum Sample:
   Verify the required sample type with the laboratory before starting the evaluation.
   i. Serum
      Most laboratories prefer to run lipid analysis using a serum specimen. Thus, this sample must be collected in a tube without anticoagulant (clot tubes, red top and SST). This sample is allowed to clot and then be centrifuged, and then the serum can be sent to the 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 then the plasma can be sent to the laboratory for analysis of total cholesterol, HDL cholesterol, and triglycerides.

IMPORTANT: The CardioChek PA analyzer is a whole blood analyzer. Serum and plasma are not appropriate samples for the CardioChek PA analyzer.

4. Evaluation Procedure

4.1. Correlation Study For Each Subject:

a. Turn on the CardioChek® PA analyzer. Insert the MEMo Chip® for the lot of Lipid Panel test strips being used. Insert a Lipid Panel test strip into the analyzer.
b. The display should read APPLY SAMPLE.
    i. If the CardioChek PA analyzer displays RUN TEST, press the Enter button to access the APPLY SAMPLE display.
c. Perform the fingerstick. Collect the sample in a PTS 40µL capillary pipette.
d. Dispense the blood sample onto the blood application window of the test strip. (The CardioChek PA analyzer will automatically begin testing the sample.)
e. Repeat steps a & b on two (2) additional CardioChek PA analyzers.
f. Gently mix the venous blood sample by inversion. Remove the stopper and insert a PTS 40µL capillary pipette into the tube to collect the blood sample.
    i. Alternatively a precision pipette can be used to transfer the blood specimens from the collection tube to the CardioChek PA analyzer.
g. Dispense the blood sample onto the blood application window of the test strip on one analyzer. (The CardioChek PA analyzer will automatically begin testing the sample.)
h. Use another PTS 40µL capillary pipette to collect the second venous sample and dispense onto the blood application window of the other analyzer. When complete, replace the stopper on the blood collection tube.
i. When the CardioChek PA analyzer displays the results (CHOL, HDL, TRIG), record on the attached data collection form.
j. Turn the used test strip over and confirm that the three reaction circles on the back side of the test strip 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 test strip or that the test strip was unevenly colored.
k. To test the next sample, press the Enter button until the display reads INSERT STRIP and repeat steps a through j.

4.2. Precision Study:

a. Select three (3) venous subject samples from those collected for the correlation study.
    i. Samples should be selected such that they display CardioChek PA results near the low, mid, and high analyte range of linearity for each analyte.

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

b. Test each sample 10 times on the CardioChek PA analyzer.
c. Record the results on the attached precision study data form.
d. To test the sample again, press the Enter button so the display reads INSERT STRIP.
e. Turn the used test strip over and confirm that the three reaction circles on the back side of the test strip 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.

5. Data Analysis

NOTE: All data submitted to the PTS, Inc. Clinical and 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® PA result and the lab result using the following formulas:
    i. CardioChek result – Laboratory result = Difference
    ii. (Difference / Laboratory result) x 100 = % Difference

NOTE: If the CardioChek PA result is greater than the laboratory result, the difference will be a positive (+) number. If the CardioChek PA 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 PA test system is performing acceptably if the mean difference for all the results is within:

Total cholesterol	±10%
HDL cholesterol	±12%
Triglycerides	±15%

5.2. Linear Regression:

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

CardioChek PA Result = slope * (Lab result) + 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 PA result and the % difference between the laboratory result and the predicted CardioChek PA result.

For the PTS Panels® 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

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

5.3. Precision Study:

a. For the 10 replicates of each subject sample, calculate the mean, standard deviation (SD), and coefficient of variation (CV).
b. The CardioChek PA test system is performing acceptably if the coefficient of variation (CVs) of total cholesterol, HDL cholesterol, and triglycerides from this precision study are <10%.

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

The collective analysis of the correlation and precision data are used to assess the CardioChek® PA test system and provide assurance that the system is giving the expected results. Bias is typically controlled in the CardioChek PA analyzer by means of the MEMo Chip® which provides a lot specific conversion of the CardioChek PA results to reference laboratory results; this is done during the routine Quality Control testing of the test strip lot. Precision reflects the variability across different lipid panel test strips as they are tested on the CardioChek PA test system. 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, and triglycerides results. It is thus important to interpret all laboratory results with this known variability in mind.

Data Collection - Correlation Study – Laboratory Reference

PTS Panels® Test Strips: Lipid Panel
CardioChek® PA Serial Number
Reference Lab Method

Lot Number: _____ Exp Date: _____
Operator Name: _____ Date: _____
Instrument: _____ Reagents: _____

Specimen: (circle) Venous Fingerstick

Seq. No	Sample Type	Sample ID	CardioChek PA			Reference Value			% difference		
			CHOL	HDL	TRIG	CHOL	HDL	TRIG	CHOL	HDL	TRIG
1	WB										
2	WB										
3	WB										
4	WB										
5	WB										
6	WB										
7	WB										
8	WB										
9	WB										
10	WB										
11	WB										
12	WB										
13	WB										
14	WB										
15	WB										
16	WB										
17	WB										
18	WB										
19	WB										
20	WB										
Average Difference											
Expected Results									**±10%**	**±12%**	**±15%**

Comments: _____
Approval: _____

Data Collection Sheet - Precision Study

PTS Panels® Test Strips: Lipid Panel
CardioChek® PA Serial Number

Lot Number: _____ Exp Date: _____
Operator Name: _____ Date: _____

Seq No.	CHOL	HDL	TRIG	Seq No.	CHOL	HDL	TRIG	Seq No.	CHOL	HDL	TRIG
1				1				1			
2				2				2			
3				3				3			
4				4				4			
5				5				5			
6				6				6			
7				7				7			
8				8				8			
9				9				9			
10				10				10			
Mean											
Standard Deviation (SD)											
Coefficient of Variation (CV)											
Expected CV	<10 %	<10 %	<10 %		<10 %	<10 %	<10 %		<10 %	<10 %	<10 %

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