CE

skyla™ HB1

skyla™ Lipid Panel

IVD

PN：800-160

For In Vitro Diagnostic Use and For Professional Use Only

Rev：C

==========

1. Intended Use

The skyla Lipid Panel used with skyla Clinical Chemistry Analyzer, is intended to be used for the quantitative determination of Blood Glucose (GLU), High-Density Lipoprotein (HDL), Total Cholesterol (TC), Triglyceride (TG) in human whole blood, plasma, or serum. The calculated values of Low-Density Lipoprotein (LDL), and Very Low-Density Lipoprotein (VLDL) can then be obtained.

2. Principles

The skyla Lipid Panel contains a total of 4 types of dried reagents located in the respective detection wells of the reagent disc. The user only needs to inject the blood specimens into the sample port of the disc, and places the disc into the analyzer. The test will be done automatically within 15 minutes. Two additional calculated values are also obtained after the test. For the detail description of disc, please refer to “skyla Clinical Chemistry Analyzer Operator’s Manual”.

Clinical Significance:

Glucose (GLU)

GLU can be used for the diagnosis of diabetes and diseases related to the carbohydrate metabolism. Diabetes, chronic pancreatitis and certain endocrine diseases may lead to hyperglycemia. Abnormal

glucose metabolism, islet cell tumors, pancreatic tumors and severe liver diseases may lead to hypoglycemia.

High-Density Lipoprotein (HDL)

HDL is an important substance that helps the human body to prevent arteriosclerosis. HDL can be used to determine the ischemic heart diseases, cerebral arteriosclerosis, stroke and other illnesses caused by excessively low HDL.

Total Cholesterol (TC)

TC test can be used to assess the metabolic state of lipids. When there is an excessive amount of TC in the serum, atherosclerosis or hypertension are a likely cause and could lead to myocardial infarction or stroke. The lipoprotein is also an important marker to determine the risk of atherosclerosis.

Triglyceride (TG)

TG test can be used to assess the metabolic state of lipids. When there is an excessive amount of TG in the serum, atherosclerosis or hypertension are a likely cause and could lead to myocardial infarction or stroke. Other possible causes of elevated TG include poorly controlled diabetes, nephrotic syndrome, hypothyroidism, hereditary hypertriglyceridemia or alcohol.

Low-Density Lipoprotein (LDL)

This parameter is calculated from HDL, TC, and TG. Excessive LDL is a warning sign of cardiovascular diseases, which in turn may cause hyperlipoproteinemia, nephrotic syndrome, obstructive hepatitis, and hypothyroidism. Excessively low LDL may lead to β hyperlipoproteinemia and liver cell failure. LDL is also an important indicator of coronary heart disease.

Very Low-Density Lipoprotein (VLDL)

VLDL is calculated from TG and is closely associated with TG. Diabetes, pancreatitis, uremia, nephritis, pregnancy, taking birth control pills, alcohol, obesity can lead to elevated VLDL value.

Method:

GLU

GLU is determined through the endpoint enzymatic reaction approach. The Sucrose is catalyzed by Hexokinase to D-Glucose-6-Phosphate (G-6-P). In the presence of NAD, G-6-PD converts G-6-P into 6- Phosphogluconate and NADH. The absorbance at the wavelength of 340 nm can be measured in the presence of NADH. The absorbance is proportional to the GLU concentration.

HDL

HDL is determined through the endpoint enzymatic reaction approach. $MnCl_2$ and Dextran Sulfate form insoluble compounds with LDL, VDL and Chylomicrons that are removed by centrifugal force. The remaining HDL is hydrolyzed by PEG-CE into Cholesterol and Fatty Acids. Cholesterol reacts with PEG-CO yielding Cholest-4-En-3-One and Peroxide (H_2O_2). The following Peroxidase reaction with H_2O_2 results in a wine-red colored product that has an absorbance at wavelength of 510 nm. The original HDL concentration is directly proportional to the absorbance maximum in this end-point reaction.

TC

TC is determined enzymatically by an endpoint reaction. It is hydrolyzed by Cholesterol Esterase (COE) into free Cholesterol and Fatty Acids. Cholesterol and NAD reacts with Cholesterol Dehydrogenase (CDH) to produce Cholest-4-En-3-One and NADH. The absorbance at the wavelength of 340 nm can be measured in the presence of NADH. The absorbance is proportional to the TC concentration.

TG

TG is determined enzymatically. Lipase converts the Triglycerides to Glycerol and Fatty Acids. In a subsequent step, Glycerol Kinase converts Glycerol into Glycerol Phosphate, which is oxidized, producing Dihydroxyacetone Phosphate and Peroxide (H_2O_2) in the process. The Peroxidase reaction with H_2O_2 results in the production of a wine-red colored product that has an absorbance maximum at 510 nm. The absorbance is proportional to the TG concentration.

Reaction pathway :

GLU

$$\text{D-Glucose + ATP} \xrightarrow{\text{Hexokinase}} \text{D-Glucose-6-Phosphate + ADP}$$

$$\text{D-Glucose-6-Phosphate + NAD} \xrightarrow{\text{G-6-PDH}} \text{6- Phosphogluconate + NADH + } H^+$$

HDL

$$\text{CM,LDL,VLDL,and HDL + Dextran Sulfate + } MnCl_2 \longrightarrow \text{HDL + Insoluble Complexes}$$

$$\text{Insoluble Complexes Reaction Curve} \xrightarrow{\text{Centrifugation}} \text{Insoluble Complexes Pelleted Against Wall of Reaction Well}$$

$$\text{HDL-Cholesterol Esters + } H_2O \xrightarrow{\text{PEG-CE}} \text{Cholesterol + Fatty Acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{PEG-CO}} \text{Cholest-4-En-3-One + } H_2O_2$$

$$H_2O_2 + \text{4-AAP + DCHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine + } H_2O$$

TC

$$\text{Cholesterol Esters + } H_2O \xrightarrow{\text{COE}} \text{Cholesterol + RCOOH}$$

$$\text{Cholesterol + } NAD^+ \xrightarrow{\text{CDH}} \text{Cholest-4-En-3-One + NADH+}H^+$$

TG

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Fatty Acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{GK}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone Phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{DCHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + H_2O$$

3. Reagents

Included:

Each panel contains dried reagent beads, dried internal QC beads and the diluent.

Reagent Composition:

Composition	Quantity/Panel
4-AAP	0.022 mg
ATP	0.07 mg
Cholesterol Dehydrogenase	0.36 U
Cholesterol Esterase	1.5 U
Cholesterol Oxidase	0.25 U
Dextran Sulfate	0.01 mg

Composition	Quantity/Panel
G6PDH	0.2 U
Glycerol Kinase	0.03 U
Hexokinase	0.1 U
L-alpha-Glycerophosphate Oxidase	0.02 U
Magnesium Chloride	0.001 mg
NAD	0.28 mg
Peroxidase	0.94 U

Reagent Storage:

- The reagent disc should be stored at 2～8℃.
- The expiry date of the reagent is printed on the outside of the sealed pouch of reagent disc. Do not use if the reagents have expired.

4. Specimen Collection and Preparation

Specimen Collection:

- Specimens suitable for skyla Lipid Panel include lithium heparinized whole blood, lithium heparinized plasma, serum and quality control solutions. The sample requirement is 200 μL. (±10μL tolerance are allowable)
- Collection, preservation and handling of specimens in accordance with local legal requirements or the standard operating procedures of your organization.

Note: Do not use specimens containing other coagulants. That would cause in incorrect test results.

Specimen Preparation:

- Before applying a sample to the reagent disc, gently rotate the sample tube up and down several times, to confirm the sample is homogeneous (evenly mixed). If the sample is whole blood, do not shake the sample container vigorously to avoid occurrence of hemolysis.

Note: 1. Perform testing within 10 minutes after applying the sample to the reagent disc.

2. The use of whole blood specimens with hematocrits (Hct) higher than 60% may affect the test results.

For further information in specimen collection and preparation, please refer to "skyla Clinical Chemistry Analyzer Operator's Manual".

5. Test Procedures

Material Preparation:

1 piece of the reagent disc of skyla Lipid Panel

Required materials not included in the panel:

The skyla Clinical Chemistry Analyzer

Sample collection container

Micropipette / Tips

Control reagents available on the market.

Test Conditions:

Test should be carry out in an environment with temperatures of 10℃~32℃. Each test will take about 15 minutes. During the test, chamber in the analyzer keeps the temperature at 37℃ for stable assay.

Test Steps:

1. Open the aluminum pouch and remove the reagent disc.
2. Remove the diluent container sealing.
3. Using a micropipette to inject 200μL of the sample into the reagent disc through the sample port.
4. Place the reagent disc to the analyzer drawer.
5. Press the "start" button on the screen to initiate testing.

For details on the operating steps and instrument setting, refer to "the skyla™ Clinical Chemistry Analyzer Operator's Manual."

Note: 1. To operate the reagent disc or instrument, please wear lab gloves and other protective gear to avoid contamination by specimen.

2. The used reagent disc, tips should be discarded as biomedical waste.
3. Testing should be performed within 20 minutes after the pouch is opened.
4. Do not place the reagent disc at the environment more than 25℃ and longer than 48 hours prior to use.
5. If the reagent disc or its package is damaged or is over the expiry date, do not use it.

6. Calibration

The barcode on every manufactured reagent disc contains all information required for calibration of the test items. The analyzer will automatically read the barcode information during testing.

7. Quality Control

External quality control materials can be used for the accuracy monitor of skyla system. The recommended frequency of QC testing is as follows. (External quality control materials are not provided by LITE-ON)

- At least every 30 days.
- Before a new batch of reagents is used for testing.
- When the analyzer is moved or the operating environment significantly changes.

8. Reference interval

The table below shows the reference interval for each test item. These ranges are provided as a reference only. It is recommended that every laboratory or test site should establish its own reference interval from its particular patient population.

Test Item	Reference Interval	Reference Interval (SI Unit)
GLU	70 – 110 mg/dL	3.9 – 6.1 mmol/L
HDL	> 40 mg/dL	> 1.0 mmol/L
TC	< 200 mg/dL	< 5.2 mmol/L
TG	< 150 mg/dL	< 1.7 mmol/L

9. Limitation

Interference studies:

1. Effect of endogenous substances

Physiological interferents in blood include hemolysis, icterus, and lipemia. For every test item, 2 Levels human serum pool supplemented with known concentrations of the endogenous substances were used for the testing. Significant interference is defined as a >10% shift in the test result.

Test Item	substance concentration with interferences of less than 10%			
	Hemolysis [Hemoglobin]	Icterus [Bilirubin (unconjugated)]	Icterus [Bilirubin (conjugated)]	Lipemia [Intralipid]
GLU	600 mg/dL	62.5 mg/dL	55.5 mg/dL	0.017%
HDL	700 mg/dL	14.97 mg/dL	0.89mg/dL	0.22%
TC	300 mg/dL	30.0 mg/dL	30.0mg/dL	0.2%
TG	315.2 mg/dL	14.6 mg/dL	2.6 mg/dL	---

2. Effect of exogenous substances

Ten exogenous substances were selected as potential interferents for the study. For every test item, human serum pool supplemented with a known concentration of the substances was used for the testing. Significant interference is defined as a >10% shift in the test result.

Substance	Test Concentration	Affected Test Item	Effect
Acetaminophen	20 mg/dL	No significant interference	
Acetylsalicylic acid	65 mg/dL	HDL	23.0% Dec.

Substance	Test Concentration	Affected Test Item	Effect
Ampicillin	5 mg/dL	TG	11.9% Inc.
		HDL	12.4% Inc.
Ascorbic acid	6 mg/dL	TG	13.4% Inc.
Caffenine	6 mg/dL	No significant interference	
Cephalothin	30 mg/dL	HDL	18.9% Dec.
Cimetidine	2 mg/dL	HDL	11.9% Dec.
Ibuprofen	50 mg/dL	No significant interference	
Salicylic acid	60 mg/dL	HDL	11.2% Dec.
Theophylline	4 mg/dL	No significant interference	

10. Performance Characteristics

Dynamic range:

The dynamic range was determined by linearity study, as follows:

Test Item	Dynamic Range	Dynamic Range (SI Unit)
GLU	30 – 600 mg/dL	1.7 – 33.3 mmol/L
HDL	20 – 75 mg/dL	0.5 – 1.9 mmol/L
TC	50 – 540 mg/dL	1.3 – 14.0 mmol/L
TG	35 – 600 mg/dL	0.4 – 6.8 mmol/L

Analytical Sensitivity:

The sensitivity (limits of quantitation) was determined according to the lowest concentration of the dynamic range which had an acceptable CV (CV<20%). The sensitivity of each test item is shown in the table below.

Test Item	Limit of Detection	Test Item	Limit of Detection
GLU	30 mg/dL	TC	50 mg/dL
HDL	20 mg/dL	TG	35 mg/dL

Precision:

Precision studies adopt serum pool of high and low concentrations as test samples. Tests are performed twice a day for a total of 20 days. Results for repeatability and reproducibility of each test item are shown in the table below.

Level 1					
Test Item	Mean	Within-Run		Total	
		SD	%CV	SD	%CV
GLU	84.7 mg/dL	1.4	**1.6**	1.4	**1.7**
HDL	56.1 mg/dL	2.1	**3.8**	2.5	**4.4**
TC	246.6 mg/dL	3.1	**1.3**	3.4	**1.4**
TG	180.6 mg/dL	2.8	**1.5**	3.3	**1.8**

Level 2					
Test Item	Mean	Within-Run		Total	
		SD	%CV	SD	%CV
GLU	274.7 mg/dL	2.4	**0.9**	3.2	**1.1**
HDL	33.8 mg/dL	1.4	**4.1**	1.6	**4.7**

Level 2					
Test Item	Mean	Within-Run		Total	
		SD	%CV	SD	%CV
TC	109.8 mg/dL	1.4	**1.3**	2.3	**2.1**
TG	96.9mg/dL	1.7	**1.8**	1.9	**1.9**

Method Comparison:

The automatic clinical chemistry analyzer in clinical laboratory was used as comparative method in the study. The tests are performed by using the same clinical serum sample for two methods. Correlation between two methods can be determined through statistical analysis.

Test Item	Correlation Coefficient (R)	Slope	Intercept	SEE	N	Sample range
GLU	0.9986	1.004	0.2	6.3	56	32 – 640 mg/dL
HDL	0.9886	0.926	3.71	2.92	40	19.2 – 82.9 mg/dL
TC	0.9814	0.988	13.1	11	41	44 – 346 mg/dL
TG	0.9886	0.990	-0.2	14	58	39 – 474 mg/dL

Matrix Comparison:

The Correlation between WB, plasma and serum was determined. The clinical sample was used in the study.

Test Item	N	Matrix type	Correlation Coefficient (R)	Slope	Intercept
GLU	15	Serum vs. Plasma	0.9831	1.002	2.040
		Plasma vs. WB	0.9851	1.060	-3.935
		WB vs. Serum	0.9911	0.941	1.619
HDL	9	Serum vs. Plasma	0.9960	1.034	-2.8
		Plasma vs. WB	0.9944	1.010	-1.2
		WB vs. Serum	0.9927	0.977	1.5
TC	15	Serum vs. Plasma	0.9923	1.032	-2.900
		Plasma vs. WB	0.9804	0.928	9.433
		WB vs. Serum	0.9897	1.043	-6.927
TG	13	Serum vs. Plasma	0.9923	1.019	-1.824
		Plasma vs. WB	0.9892	1.042	-2.049
		WB vs. Serum	0.9948	0.942	3.678

11. Reference

1. Clinical and Laboratory Standards Institute. Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. CLSI document EP07-A2. Robert J. McEnroe: 2005.
2. Clinical and Laboratory Standards Institute. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. NCCLS document EP06-A. Dan Tholen: 2003.
3. Clinical and Laboratory Standards Institute. Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. NCCLS document EP17-A. Daniel W. Tholen: 2004.
4. Clinical and Laboratory Standards Institute. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. NCCLS document EP05-A2. Jan S. Krouwer: 2004.
5. Clinical and Laboratory Standards Institute. Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition. NCCLS document EP09-A2. Jan S. Krouwer: 2002.

Symbol Index			
REF	Catalogue number		Consult instruction for use
LOT	Batch code		Use by
	Manufacturer	EC REP	Authorized representative in the European Community
IVD	In Vitro diagnostic medical device	CE	CE mark
	Temperature limitation		Caution
	Do not reuse		

LITE-ON Technology Corporation H.S.P.B.

No. 8, Dusing Road, Hsinchu Science Park
Hsinchu, Taiwan

EC REP

MT Promedt Consulting GmbH
Altenhofstr. 80
D-66386 St. Ingbert
Germany

Customer service/Technical support ：+886-3-611-8511

Email ：support@skyla.com

Website ：www.skyla.com

Issue date: 20 Dec 2013

Revise date: 18 APR 2016

PN: 7B25000057HD

LITE-ON Technology Corp.