CE

skyla™ HB1

skyla™ Liver Panel

IVD

PN：800-120

Rev：E

For In Vitro Diagnostic Use and For Professional Use Only

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1. Intended Use

The skyla Liver Panel used with skyla Clinical Chemistry Analyzer, is intended to be used for the quantitative determination of Albumin (ALB), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT/GPT), Aspartate Aminotransferase (AST/GOT), Direct Bilirubin (DBIL), Gamma-Glutamyl Transpeptidace (GGT), Blood Glucose (GLU), Total Bilirubin (TBIL), Total Protein (TP) in human whole blood, plasma, or serum. The calculated values of Albumin/Globulin Ratio (A/G Ratio), Globulin (GLOB), and Indirect Bilirubin (IBIL) can then be obtained.

2. Principles

The skyla Liver Panel contains a total of 9 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. Three 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:

Albumin (ALB)

ALB is the major protein component of normal human serum, accounting for more than 50% of the total protein. It plays an important role in the regulation of the osmotic blood pressure. Abnormal ALB values may be caused by dehydration, malnutrition, nephrotic syndrome or liver dysfunction.

Alkaline Phosphatase (ALP)

ALP is an indicator for hepatobiliary or bone disorder. Increased ALP activity can be associated with disorders include of Hodgkin's disease, congestive heart failure, ulcesative colitis, regional enteritis, and intraabdominal bacterial.

Alanine Aminotransferase (ALT/GPT)

ALT is one of the indicators of liver function. Acute and chronic hepatitis, drug induced liver injury, fatty liver, cirrhosis, myocardial infarction, myocarditis and biliary diseases can lead to elevated ALT activity.

Aspartate Aminotransferase (AST/GOT)

AST is one of the indicators of liver function. Increased AST activity can be associated with medical conditions involving heart, liver, kidney or pancreas. Myocardial infarctions cause an increase of AST levels in the blood within 6-8 hours, gradually returning to normal levels after 48-60 hours. Hepatitis or other hepatobiliary diseases can also increase AST activity.

Direct bilirubin (DBIL)

DBIL can be used for the diagnosis of obstructive jaundice, congenitalnon-hemolytic jaundice and indirect post-hepatitis hyperbilirubinemia.

Gamma-Glutamyl Transpeptidace (GGT)

GGT is an enzyme secreted by the gall bladder. Determination of GGT levels can provide valuable clues in the diagnosis of liver diseases, alcohol poisoning or kidney failure.

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.

Total bilirubin (TBIL)

TBIL can be used for the diagnosis of acute hepatitis, chronic hepatitis, cirrhosis, cholangitis, cholelithiasis, cholecystitis, hepatobiliary diseases and hemolytic anemia.

Total Protein (TP)

TP is an indicator for the liver function and kidney diseases. Elevated TP could be caused by dehydration or increased immunoglobulin levels. And TP reduction may occur in the disorders include malnutrition, nephrotic syndrome, various liver diseases and malignant tumors.

Albumin/Globulin Ratio (A/G Ratio)

The A/G Ratio is the ALB and GLOB ratio. It is used to assess liver function and is an important indicator for the diagnosis of viral hepatitis and cirrhosis.

Globulin (GLOB)

It is calculated from TP and ALB. It is used to assess liver function.

Indirect bilirubin (IBIL)

It is calculated from TBIL and DBIL. IBIL is important for the diagnosis of hepatocellular jaundice, hemolytic jaundice and other hepatobiliary diseases.

Method:

ALB

ALB is determined through the endpoint color reaction method. When binding to Bromocresol Green (BCG), it forms a yellow-green complex. The absorbance at a wavelength of 600 nm can be measured. The amount of ALB in the sample is proportional to the bound ALB.

ALP

ALP activity is enzymatically determined. *p*-Nitrophenyl Phosphate that is hydrolyzed by ALP into a yellow colored product *p*-Nitrophenol which has an absorbance at a wavelength of 405 nm. The rate of the reaction is directly proportional to the enzyme activity.

ALT

ALT activity is enzymatically determined. ALT catalyses the reaction of Alanine with α-Ketoglutarate, converting them into Glutamate and Pyruvate. In the presence of NADH, Lactate Dehydrogenase converts Pyruvate into Lactate. In the course of the reaction NADH is oxidized to NAD. The decrease of NADH absorbance is measured at a wavelength of 340 nm and is proportional to ALT activity.

AST

AST activity is enzymatically determined. When the test sample reacts with the substrate-enzyme reagent, AST converts L-Aspartic acid and α-Ketoglutarate into Monosodium Glutamate and Amide Acetate. Amide Acetate is subsequently converted into Malate by Malate Dehydrogenase while NADH undergoes oxidation to NAD. The decrease of NADH absorbance is measured at a wavelength of 340 nm and is proportional to AST activity.

DBIL

DBIL is determined by vandate oxidation. In a pH3 buffer solution, DBIL spontaneously undergoes oxidation forming Biliverdin. The DBIL concentration is measured by the decline of the specific yellow absorbance at wavelength of 450 nm in the presence of Vanadium.

GGT

GGT is enzymatically determined. GGT catalyzes the reaction between L-γ-Glutamyl-3-Carboxy-4-Nitroanilide and Gly-Gly, and cause the formation of L-γ-Glutamyl-Glycylgycine and 5-Amino-2-Nitrobenzoate with yellow color. The rate of liberation of 5-Amino-2 Nitrobenzoate is directly related to the GGT activity in the sample and is quantitated by measuring the increase in absorbance at wavelength of 405 nm.

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.

TBIL

TBIL is determined by the vandate oxidation. In a pH3 buffer solution TBIL undergoes oxidation forming Biliverdin. The TBIL content is measured by the decline of the specific yellow absorbance at 450 nm in the presence of Vanadium.

TP

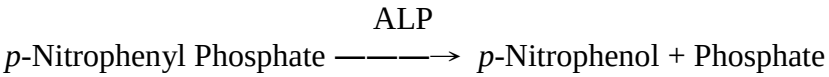
TP is determined by the Biuret method. The peptide bonds of the protein react with Copper ions in an alkaline environment and form a purple compound. The color development is proportional to the original TP concentration and is measured at wavelength of 546 nm.

Reaction pathway :

ALB

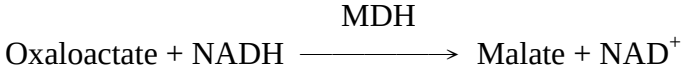
Albumin + BCG $\longrightarrow$ Albumin-BCG Complex

ALP



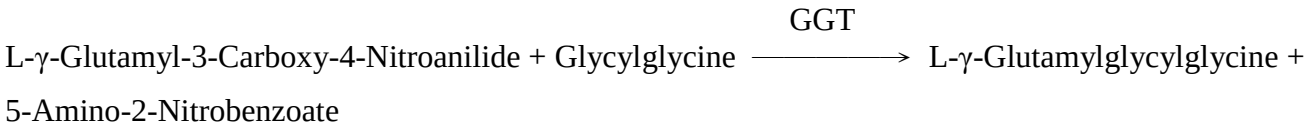
ALT

AST



DBIL

GGT



GLU

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

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

TBIL

$$\text{Bilirubin + Surfactant + } VO_3^{-} \longrightarrow \text{Biliverdin}$$

TP

$$\text{Total protein + } Cu^{2+} \xrightarrow{\text{Alkali}} \text{Cu-Protein Complex}$$

3. Reagents

Included:

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

Reagent Composition:

Composition	Quantity/Panel
4-Nitrophenyl Phosphate Disodium Salt	0.1 mg
ATP	0.04 mg
Bromocresol Green	5.4 μg
Copper Sulfate	0.1 mg
G6PDH	0.2 U
Glycylglycine	0.38 mg
Hexokinase	0.1 U

Composition	Quantity/Panel
Lactate Dehydrogenase	0.3 U
L-Alanine	0.3 mg
L-Aspartic Acid	1 mg
L-γ-Glutamyl-3-Carboxy-4-Nitroanilide	0.1 mg
Malate Dehydrogenase	0.04 U
NAD	0.1 mg
NADH	0.05 mg
Sodium Metavanadate	0.02 mg
α-Ketoglutaric Acid	0.2 mg

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 Liver 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 Liver 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)
ALB		3.5 – 5.3 g/dL	35 – 53 g/L
ALP		< 108 U/L	< 108 U/L
ALT		< 40 U/L	< 40 U/L
AST		< 42 U/L	< 42 U/L
DBIL		< 0.4 mg/dL	< 6.8 μmol/L
GGT	Male	< 73 U/L	< 73 U/L

Test Item	Reference Interval	Reference Interval (SI Unit)
Female	< 38 U/L	< 38 U/L
GLU	70 – 110 mg/dL	3.9 – 6.1 mmol/L
TBIL	< 1.2 mg/dL	< 20.5 μmol/L
TP	6.0 – 8.3 g/dL	60 – 83 g/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]
ALB	147.6 mg/dL	62.5 mg/dL	57.5 mg/dL	0.11%
ALP	600 mg/dL	31.7 mg/dL	57.5 mg/dL	0.02%
ALT	290 mg/dL	43.5 mg/dL	22.3 mg/dL	0.02%
AST	3.3 mg/dL	22.9 mg/dL	47.2 mg/dL	0.05%
DBIL	400 mg/dL	---	---	0.03%
GGT	265.4 mg/dL	33.4 mg/dL	10.5 mg/dL	0.2%
GLU	600 mg/dL	62.5 mg/dL	55.5 mg/dL	0.017%

Test Item	substance concentration with interferences of less than 10%			
	Hemolysis [Hemoglobin]	Icterus [Bilirubin (unconjugated)]	Icterus [Bilirubin (conjugated)]	Lipemia [Intralipid]
TBIL	293.2 mg/dL	---	---	0.03%
TP	157.2 mg/dL	62.5 mg/dL	57.5 mg/dL	0.07%

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	DBIL	11.1% Dec.
Acetylsalicylic acid	65 mg/dL	ALP	10.2% Dec.
Ampicillin	5 mg/dL	No significant interference	
Ascorbic acid	6 mg/dL	No significant interference	
Caffenine	6 mg/dL	No significant interference	
Cephalothin	30 mg/dL	No significant interference	
Cimetidine	2 mg/dL	No significant interference	
Ibuprofen	50 mg/dL	No significant interference	
Salicylic acid	60 mg/dL	No significant interference	
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)
ALB	1.0 – 6.0 g/dL	10 – 60 g/L
ALP	41 – 2000 U/L	41 – 2000 U/L
ALT	20 – 1100 U/L	20 – 1100 U/L
AST	20 – 1000 U/L	20 – 1000 U/L
DBIL	0.1 – 15 mg/dL	1.7 –256.6 μmol/L
GGT	10 – 1500 U/L	10 – 1500 U/L
GLU	30 – 600 mg/dL	1.7 – 33.3 mmol/L
TBIL	0.4 – 30 mg/dL	6.8 –513.1 μmol/L
TP	1.5 – 10 g/dL	15 – 100 g/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
ALB	1.0 g/dL	GGT	10 U/L
ALP	41 U/L	GLU	30 mg/dL
ALT	20 U/L	TBIL	0.4 mg/dL

Test Item	Limit of Detection	Test Item	Limit of Detection
AST	20 U/L	TP	1.5 g/dL
DBIL	0.1 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
ALB	4.89 g/dL	0.09	**1.8**	0.09	**1.8**
ALP	71.9 U/L	1.7	**2.3**	1.7	**2.3**
ALT	54.0 U/L	1.6	**3.0**	1.7	**3.1**
AST	43.7 U/L	1.8	**4.1**	2.0	**4.5**
DBIL	0.5 mg/dL	0.02	**3.2**	0.02	**3.2**
GGT	51.2 U/L	1.7	**3.3**	1.7	**3.3**
GLU	84.7 mg/dL	1.4	**1.6**	1.4	**1.7**
TBIL	1.41 mg/dL	0.02	**1.1**	0.07	**4.7**
TP	6.65 g/dL	0.07	**1.0**	0.07	**1.0**

Level 2					
Test Item	Mean	Within-Run		Total	
		SD	%CV	SD	%CV
ALB	2.56 g/dL	0.05	**2.1**	0.06	**2.2**
ALP	423.7 U/L	9.2	**2.2**	10.3	**2.4**
ALT	194.6 U/L	6.1	**3.1**	6.3	**3.2**
AST	202.3 U/L	3.1	**1.5**	3.8	**1.9**
DBIL	2.21 mg/dL	0.02	**0.7**	0.04	**1.6**

Level 2					
Test Item	Mean	Within-Run		Total	
		SD	%CV	SD	%CV
GGT	141.2 U/L	3.3	**2.3**	3.9	**2.8**
GLU	274.7 mg/dL	2.4	**0.9**	3.2	**1.1**
TBIL	4.58 mg/dL	0.1	**2.3**	0.11	**2.4**
TP	4.16 g/dL	0.06	**1.4**	0.06	**1.5**

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
ALB	0.9850	1.008	-0.015	0.148	52	1.63 – 5.34 g/dL
ALP	0.9923	0.997	-0.5	22	48	45 – 888 U/L
ALT	0.9995	1.019	0.9	5.1	44	4 – 807 U/L
AST	0.9987	1.008	2.7	7	44	2 – 851 U/L
DBIL	0.9910	1.000	0.036	0.243	46	0.02 – 11.6 mg/dL
GGT	0.9990	0.999	0.6	5.7	54	5 – 1224 U/L
GLU	0.9986	1.004	0.2	6.3	56	32 – 640 mg/dL
TBIL	0.9949	1.001	0.096	0.501	47	0.11 – 25.98 mg/dL
TP	0.9911	0.999	-0.008	0.202	52	2.36 – 9.34 g/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
ALB	5	Serum vs. Plasma	0.9949	1.000	-0.04
		Plasma vs. WB	0.9999	1.005	-0.10
		WB vs. Serum	0.9961	0.996	0.14
ALP	5	Serum vs. Plasma	0.9986	0.996	-6.2
		Plasma vs. WB	0.9998	0.962	0.6
		WB vs. Serum	0.9977	1.044	5.6
ALT	11	Serum vs. Plasma	0.9991	0.984	-0.261
		Plasma vs. WB	0.9980	0.996	-1.003
		WB vs. Serum	0.9989	1.020	1.288
AST	12	Serum vs. Plasma	0.9982	0.954	-0.074
		Plasma vs. WB	0.9962	1.047	-0.732
		WB vs. Serum	0.9980	1.001	0.805
DBIL	10	Serum vs. Plasma	0.9987	1.033	0.013
		Plasma vs. WB	0.9990	1.047	0.018
		WB vs. Serum	0.9983	0.925	-0.029
GGT	11	Serum vs. Plasma	0.9980	0.912	-8.942
		Plasma vs. WB	0.9996	1.078	-3.013
		WB vs. Serum	0.9987	1.017	-6.726
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
TBIL	13	Serum vs. Plasma	0.9939	1.030	0.003
		Plasma vs. WB	0.9980	1.031	0.062
		WB vs. Serum	0.9948	0.941	-0.061
TP	15	Serum vs. Plasma	0.9926	0.967	0.325
		Plasma vs. WB	0.9965	1.038	-0.188
		WB vs. Serum	0.9960	0.996	-0.148

11. Reference

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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		

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