skyla

Liver Plus Panel

PN：900-180

For Veterinary In Vitro Diagnostic Use Only **Rev：D**

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1. Intended Use

The skyla Liver Plus Panel used with skyla Analyzer, is intended to be used for the quantitative determination of Albumin (ALB), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Blood Urea Nitrogen, (BUN), Total bilirubin (TBIL), Total Protein (TP), Total Cholesterol (CHOL), γ-Glutamyl Transpeptidase (GGT) and Bile Acid (BA) in animal whole blood, plasma, or serum. The calculated values of Globulin (GLOB), Albumin/Globulin Ratio (A/G Ratio) and UREA can then be obtained.

2. Principles

The skyla Liver Plus Panel contains a total of 10 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 then 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 Analyzer Operator’s Manual”.

Clinical Significance:

Albumin (ALB): ALB is one of the indicators for kidney function, liver function and dehydration.

Alkaline phosphatase (ALP): ALP is one of the indicators for liver and biliary related diseases.

Alanine Aminotransferase (ALT): ALT is used to detect pet viral hepatitis, cirrhosis, and the degree of liver injury and related diseases.

Aspartate Aminotransferase (AST): AST is a marker to examine hepatobiliary diseases and the degree of myocardium injury.

Blood Urea Nitrogen (BUN): BUN is one of the important markers for diagnosis and prognosis tracking of kidney diseases

Total bilirubin (TBIL): TBIL can be used for the diagnosis of obstructive liver diseases and hepatobiliary diseases.

Total Cholesterol (CHOL): CHOL test can be used to assess the metabolic state of lipids.

Total Protein (TP): TP is an indicator for function of liver synthesis and the degree of protein-losing caused by kidney diseases.

γ-Glutamyl Transpeptidase (GGT): GGT can be used for the diagnosis of liver disease, primary and secondary liver tumors.

Bile Acid (BA): BA can be used for the diagnosis of liver disease.

Globulin (GLOB): GLOB is calculated from TP and ALB and it is used to assess liver function.

Albumin/Globulin Ratio (A/G Ratio): The A/G Ratio is the ALB and GLOB ratio. It is used to assess liver function.

UREA：UREA is synthesized in the liver and secreted by the kidneys. Urea is the end product of protein nitrogen metabolism and is the primary vehicle for removing toxic ammonia from the body. The analysis of urea is an important clinical test for renal disease and dysfunction.

Method:

ALB

ALB is determined through the endpoint chemical reaction method. When ALB is 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 alanine with α-Ketoglutarate, and converts 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.

BUN

BUN is enzymatically determined. Urea undergoes a Urease catalyzed hydrolysis, thus producing

Ammonia and Carbon Dioxide. In a Glutamate Dehydrogenase (GLDH) catalyzed reaction, Ammonia reacts with 2-Oxoglutarate yielding L-Glutamate. In the process of this reaction, β-Nicotinamide Adenine Dinucleotide (NADH) is oxidized to β-Nicotinamide Adenine Dinucleotide (NAD^+) which in turn undergoes a color reaction. The rate of change of absorbance at a wavelength of 340 nm is measured and proportional to the BUN concentration.

TBIL

TBIL is determined by the vanadate oxidation method. In a pH3 buffer system, TBIL undergoes oxidation forming Biliverdin. The absorbance at a wavelength of 450 nm is measured and proportional to the total bilirubin concentration in the sample.

CHOL

CHOL 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.

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 a wavelength of 546 nm.

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.

BA

BA is enzymatically determined. In the presence of Thio-NAD^+, Bile acids reacts with enzyme 3-α-hydroxysteroid dehydrogenase (3-α-HSD) to form Oxidized bile acids and Thio-NADH. The enzyme cycling occurs, when NADH is present in the reaction, 3-α-HSD convert Oxidized bile acids back to Bile acids. The formation rate of Thio-NADH is proportional to the BA concentration in the sample. BA concentration is quantitated by measuring the absorbance at wavelength of 405 nm.

Reaction pathway :

ALB

Albumin + BCG $\longrightarrow$ Albumin-BCG Complex

ALP

$$\textit{p}\text{-Nitrophenyl Phosphate} \xrightarrow{\text{ALP}} \textit{p}\text{-Nitrophenol} + \text{Phosphate}$$

ALT

$$\text{L-Alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+ + H_2O$$

AST

$$\text{L-Asparate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloactate} + \text{L-Glutamate}$$

$$\text{Oxaloactate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + NAD^+$$

BUN

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{2-Oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + H_2O + NAD^+$$

TBIL

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

CHOL

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

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

TP

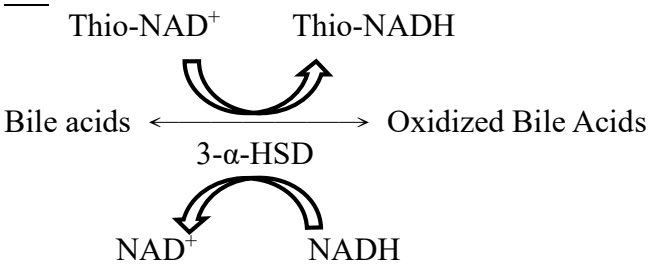
$$\text{Total protein} + Cu^{2+} \xrightarrow{\text{Alkali}} \text{Cu-Protein Complex}$$

GGT

$$\text{L-}\gamma\text{-Glutamyl-3-Carboxy-4-Nitroanilide} + \text{Glycylglycine} \xrightarrow{\text{GGT}} \text{L-}\gamma\text{-Glutamylglycylglycine} +$$

5-Amino-2-Nitrobenzoate

BA



3. Reagents

Included:

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

Reagent Composition:

Composition	Quantity/Panel
Bromocresol Green	5.4 µg
Lactate Dehydrogenase	0.3 U
Malate Dehydrogenase	0.04 U
Urease	0.12 U
Glutamate Dehydrogenase	0.01 U
Cholesterol Esterase	2.4 U
α-Hydroxysteroid Dehydrogenase	0.02 U
Cholesterol Dehydrogenase	0.5 U
Sodium azide	1.74 µg
Sodium Metavanadate	0.01 mg
Brij	1 UL
Zinc sulfate	0.001 ml
Sodium hydroxide	0.12 mg
Copper Sulfate	0.142 mg
Glycylglycine	0.38 mg
L-Aspartic Acid	0.5 mg
Citric acid	242.1 µg
TRIS Base	1.8 mg
L-Alanine	0.3 mg
Magnesium acetate	16.1 µg
Sodium citrate dehydrate	370.6 µg
α-Ketoglutaric Acid	0.15 mg
L-γ-Glutamyl-3-Carboxy-4-Nitroanilide	0.1 mg
α-Ketoglutaric acid disodium salt dehydrate	0.34 mgG
4-Nitrophenyl phosphate disodium salt	0.1 mG

Composition	Quantity/Panel
NAD	0.1 mg
NADH	0.1 mg
THIO-NAD	0.02 mg

Reagent Storage:

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

4. Specimen Collection and Preparation

Specimen Collection:

- Specimens suitable for skyla Liver Plus Panel include lithium heparinized whole blood, lithium heparinized plasma, serum and quality control materials. The sample requirement is 200 μL. (± 10 μL tolerance are allowable)
- If applicable, local regulatory or standard operating procedures of your organization should be followed for the collection, preservation and handling of specimens.

Note: Do not use specimens containing other coagulants. That would cause an 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.

Note: For further information in specimen collection and preparation, please refer to “skyla Analyzer Operator’s Manual”

5. Test Procedures

Material Preparation:

1 piece of the reagent disc of skyla Liver Plus Panel

Required materials not included in the panel:

skyla Analyzer

Sample collection container

Micropipette / Tips

Test Conditions:

Test should be carried out in an environment with temperatures of 10°C~32°C. Each test will take about 15 minutes. During the test, chamber in the analyzer keeps the temperature at 37°C 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 200 μL of the sample into the reagent disc through the sample port.
4. Press the “start” button on the screen to initiate testing.
5. Place the reagent disc to the analyzer drawer, and press the “ok” button on the screen to analysis.

For details on the operating steps and instrument setting, please refer to “skyla 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 and tips should be discarded as biomedical waste, and treat according to the local legal requirements.
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°C 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

- Please refer to the instruction manual for the preparation and use of quality control materials. For discrepancy results, the confirmatory test was suggested to carry out with the automated laboratory analyzer, or to contact with our technical support.
- External quality control materials can be used for the accuracy monitor of skyla system. The recommended frequency of QC testing is as follows, otherwise please follow local legal requirements or the standard operating procedures of your organization
  - At least every 30 days.
  - Before a new batch of reagents is used for testing.
  - When the analyzer was moved or the operating environment significantly changed.

8. Reference interval

The table below shows the reference interval for each test item. It is recommended that every laboratory or test site should establish its own reference interval from its patient population.

Test Item		Reference Interval		Reference Interval (SI Unit)	
ALB	Canine	2.6 - 4.6	g/dL	26 - 46	g/L
	Feline	2.5 - 4.6	g/dL	25 - 46	g/L
ALP	Canine	0 - 212	U/L	0 - 212	U/L
	Feline	0 - 111	U/L	0 - 111	U/L
ALT	Canine	0 - 88	U/L	0 - 88	U/L
	Feline	0 - 116	U/L	0 - 116	U/L
AST	Canine	0 - 50	U/L	0 - 50	U/L
	Feline	0 - 48	U/L	0 - 48	U/L
BUN	Canine	6.0-26.0	mg/dL	2.1 - 9.3	mmol urea/L
	Feline	13.0-37.0	mg/dL	4.6 - 13.2	mmol urea/L
TBIL	Canine	0.0 – 0.9	mg/dL	0.0 – 15.0	μmol/L
	Feline	0.0 – 0.9	mg/dL	0.0 – 15.0	μmol/L
CHOL	Canine	110-320	mg/dL	2.8 – 8.3	mmol/L
	Feline	54-220	mg/dL	1.4 – 5.7	mmol/L
TP	Canine	5.2 - 8.2	g/dL	52 - 82	g/L
	Feline	5.7 - 8.9	g/dL	57 - 89	g/L
GGT	Canine	<10	U/L	<10	U/L
	Feline	<10	U/L	<10	U/L

Test Item		Reference Interval		Reference Interval (SI Unit)	
BA	Canine	<25	μmol/L	<25	μmol/L
	Feline	<25	μmol/L	<25	μmol/L
	<5.0 μmol/L Fasting 5.0-15.0 μmol/L 2Hrs Postprandial >25μmol/L Reduced Liver Function				

9. Limitation

Physiological interferences in blood include hemolysis, icterus, and lipemia. For every test item, 2 Levels serum pool supplemented with known concentrations of the endogenous substances were used for the testing. Significant interference is defined as a >20% shift in the test result. (Note: Highest tested concentration for Hemoglobin: 600 mg/dL; Bilirubin (unconjugated): 62.5 mg/dL, Bilirubin (conjugated): 57.5 mg/dL; Intralipid: 0.55%)

Test Item	Substance concentration with interferences of less than 20%			
	Hemoglobin	Bilirubin (unconjugated)	Bilirubin (conjugated)	Intralipid
ALB	300 mg/dL	62.5 mg/dL	57.5 mg/dL	0.2%
ALP	600 mg/dL	25.9 mg/dL	57.5 mg/dL	0.1%
ALT	500 mg/dL	34.5 mg/dL	28.4 mg/dL	0.1%
AST	300 mg/dL	42.1 mg/dL	22.3 mg/dL	0.1%
BUN	500 mg/dL	42.1 mg/dL	29.3 mg/dL	0.43%
TBIL	600 mg/dL	---	---	0.1%
CHOL	300 mg/dL	30.0 mg/dL	30.0 mg/dL	0.2%
TP	300 mg/dL	62.5 mg/dL	57.5 mg/dL	0.2%
GGT	400 mg/dL	36.7 mg/dL	26.3 mg/dL	0.1%
BA	200 mg/dL	50.4 mg/dL	26.6 mg/dL	0.2%

10. Performance Characteristics

Dynamic range:

The dynamic range for each test item showed as below.

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
BUN	2.0 - 140	mg/dL	0.7-50.0	mmol urea/L
TBIL	0.4 - 30.0	mg/dL	6.8 - 513.1	μmol/L
CHOL	50-540	mg/dL	1.3 – 14.0	mmol/L
TP	1.5 - 10.0	g/dL	15 - 100	g/L
GGT	10-1500	U/L	10-1500	U/L
BA	5.0-140	μmol/L	5.0-140	μmol/L

Method Comparison:

The SIMENS ADVIA 1800 was used as comparative method in the study. The tests are performed by using the same clinical serum sample for two methods.

Marker		R^2	Slope	Intercept	Sample No.	Sample Range
ALB	Canine	0.9848	0.9999	0.0000	38	2.7-5.9 g/dL
	Feline	0.9676	1.0000	0.0000	38	3.1-6.4 g/dL
ALP	Canine	0.9626	0.9999	-0.0059	32	53-1246 U/L
	Feline	0.9581	0.9998	-0.0010	32	24-263 U/L
ALT	Canine	0.9872	0.9934	-2.4272	32	28-284 U/L
	Feline	0.9951	1.0290	0.2758	32	31-243 U/L
AST	Canine	0.9990	0.9968	0.7497	38	22-803 U/L
	Feline	0.9997	1.0033	-0.9437	38	22-891 U/L
BUN	Canine	0.9967	0.9843	0.6679	41	9.7-128.4 mg/dL
	Feline	0.9923	1.0067	-0.7677	40	17.5-126.9 mg/dL
TBIL	Canine	0.9966	0.9866	0.2672	23	0.1-31.2 mg/dL
	Feline	0.9954	0.9965	0.0687	25	0.1-31.2 mg/dL
CHOL	Canine	0.9944	0.9115	2.840	12	98-310 mg/dL
	Feline	0.9899	1.0557	-10.199	15	84-220 mg/dL
TP	Canine	0.9603	0.9999	0.0000	38	5.2-9.5 g/dL
	Feline	0.9883	0.9999	0.0000	38	6.3-10.3 g/dL
GGT	Canine	0.9992	1.0014	-0.5713	28	17-1861 U/L
	Feline	0.9988	1.0027	0.0039	12	27-1647 U/L
BA	Canine	0.9878	0.9349	0.6227	21	8.8-137 U/L
	Feline	0.9924	0.9848	-0.7697	20	9.1-131 U/L

Symbol Index			
REF	Catalogue number		Consult instruction for use
LOT	Batch code		Use by
	Manufacturer	CE	CE mark
	Temperature limitation		Caution
	Do not reuse	Σ	Sufficient for

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