skyla

Renal Panel

PN：900-110

For Veterinary In Vitro Diagnostic Use Only

Rev：J

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1. Intended Use

The skyla Renal Panel used with skyla Analyzer, is intended to be used for the quantitative determination of Albumin (ALB), Blood Urea Nitrogen, (BUN), Creatinine (CREA), Calcium (Ca), Phosphorus (PHOS), Sodium (Na), Potassium (K), Chloride (Cl), and Total Carbon Dioxide (tCO_2) in animal whole blood, plasma, or serum. The calculated values of Blood Urea Nitrogen/Creatinine Ratio (B/C Ratio), Sodium/Potassium Ratio (Na/K Ratio) and Corrected Calcium(C-Ca), UREA and Anion Gap (AGap) can then be obtained.

2. Principles

The skyla Renal 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 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.

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

Creatinine (CREA): CREA is a marker to examine renal functions.

Calcium (Ca): Ca can be used to detect parathyroid-related、bone diseases, chronic kidney diseases and tetany of vitamin D deficiency.

Phosphate (PHOS): PHOS is an indicator for kidney diseases, hypothyroidism, and malnutrition.

Sodium (Na): Na is one of indicators for fluid and electrolyte balance. It can be used to evaluate the disorders of vomiting, diarrhea, dehydration and Addison's disease.

Potassium (K): K is one of indicators for fluid and electrolyte balance. It can be used to evaluate the disorders of vomiting, diarrhea, dehydration and Addison's disease.

Chloride (Cl): Cl is one of indicators for fluid and electrolyte balance. It can be used to evaluate the disorders of vomiting, diarrhea, dehydration and renal failure.

Total Carbon Dioxide (tCO_2): tCO_2 in blood includes carbon dioxide, bicarbonate, carbonate, and carbonic acid. It is an indicator for metabolic acidosis or metabolic alkalosis.

Anion gap (AGap) : The anion gap is the difference between certain measured cations (Sodium Na+) and the measured anions (Chloride Cl- and Total carbon dioxide tCO2) in serum and plasma. Calculating the anion gap is clinically useful in the differential diagnosis of acid-base disorders.

Blood Urea Nitrogen/ Creatinine Ratio (B/C Ratio): The B/C Ratio may indicate the degree of kidney injury and azotemia.

Corrected Calcium (C-Ca): C-Ca is calculated from Ca and ALB and it is used to assess Hypocalcaemia

Sodium / Potassium Ratio (Na/K Ratio): Na/K Ratio may indicate the kidney stress, hyper-aldosteronism and Addison's disease.

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 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.

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.

CREA

CREA is determined through the endpoint enzymatic reaction approach. Creatinine Amidohydrolase hydrolyzes CREA to Creatine. Then Creatine is converted into Sarcosine through catalysis of Creatine Amidinohydrolase. Furthermore, Sarcosine Oxidase oxidizes Sarcosine, yielding Glycine, Formalehyde and Peroxide (H_2O_2) in the process. The enzyme Peroxidase processes Hydrogen Peroxide, 2,4,6-3 Hydroxy-Benzoic Acid (TBHBA) and 4-Aminoantipyrine (4-AAP), forming a Quinoneimine dye as a product. The dye formation is measured at a wavelength of 546 nm and is

proportional to the amount of CREA in the sample.

Ca

Ca is determined through the endpoint chemical reaction approach. Calcium reacts with Arsenazo III and form a purple-colored complex. The complex formation is measured at a wavelength of 650 nm and is proportional to the amount of Ca in the sample.

PHOS

PHOS is enzymatically determined. By going through a series of enzymatic reactions with Sucrose Phosphorylase, Phosphoglucomutase, and Glucose-6-Phosphate Dehydrogenase, PHOS forms 6-Phosphogluconate and NADH. And NADH is measured at a wavelength of 340 nm and is proportional to the amount of PHOS in the sample.

Na

Na is enzymatically determined. By going through the activation of β-Galactosidase with Na ion, o-Nitrophenyl-β-Galactopyranoside (ONPG) is further catalyzed by activated β-Galactosidase, form o-Nitrophenol and Galactose. The absorbance caused by o-Nitrophenol is measured at a wavelength of 405 nm and is proportional to the amount of Na in the sample.

K

K is enzymatically determined. Pyruvate Kinase (PK) dephosphorylates Phosphoenolpyruvate (PEP) to form Pyruvate. Then the Pyruvate converts to Lactate under catalysis of Lactate Dehydrogenase (LDH). At the same time, NADH is oxidized to 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 potassium in the sample.

Cl

Cl is enzymatically determined. Chloride will bind to Amylase and consequently lead to reactivation of the enzyme. Amylase will then convert a synthetic substrate α-(2-Chloro-4-Nitrophenyl)-β-1,4- Galactopyranosylmaltoside (Gal-G2-α-CNP) to 2-chloro-4-nitrophenol (CNP). Its formation and absorption at a wavelength of 405 nm is proportional to the amount of Chloride in the sample.

tCO_2

tCO_2 is enzymatically determined. It converts all forms of carbon dioxide (CO_2) toward bicarbonate (HCO_3^-), and phosphoenolpyruvate carboxylase (PEPC) makes HCO_3^- react with Phosphoenolpyruvate (PEP) to form oxaloacetate and phosphate. Malate dehydrogenase (MDH) converted nicotinamide adenine dinucleotide (NADH) to NAD^+ and malate in the presence of oxaloacetate. The rate of conversion in absorbance 340 nm is directly proportional to the amount of tCO_2 in the sample.

Reaction pathway:

ALB

$$\text{Albumin} + \text{BCG} \longrightarrow \text{Albumin-BCG Complex}$$

BUN

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

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

CREA

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatinine Amidohyrolase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{Creatine Amidohyrolase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{Sarcosine Oxidase}} \text{Glycine} + \text{Formaldehyde} + H_2O_2$$

$$H_2O_2 + \text{TBHBA} + \text{4-AAP} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine Dye} + H_2O$$

Ca

$$Ca^{2+} + \text{ArsenazoIII} \longrightarrow Ca^{2+}\text{- ArsenazoIII Complex}$$

PHOS

$$\text{Sucrose} + \text{Pi} \xrightarrow{\text{SP}} \alpha\text{-D-Glucose-1-Phosphate} + \text{D-Fructose}$$

$$\alpha\text{ -D-Glucose-1-Phosphate} \xrightarrow{\text{PGM}} \alpha\text{ -D-Glucose-6-Phosphate}$$

$$\alpha\text{ -D-Glucose-6-Phosphate} + NAD^+ \xrightarrow{\text{G6PDH}} \text{6-Phospho-D-Gluconate} + \text{NADH} + H^+$$

Na

$$\beta\text{-Galactosidase} + \text{ONPG} \xrightarrow{Na^+} \text{Galactose} + \text{o-Nitrophenol}$$

K

$$\text{ADP} + \text{PEP} \xrightarrow{K^+\text{, PK}} \text{Pyruvate} + \text{ATP}$$

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

Cl

$$\text{EDTA-Ca}^{2+} + \alpha\text{-Amylase} \xrightarrow{Cl^-} \text{EDTA} + \alpha\text{-Amylase-Ca}^{2+}$$

$$\text{Gal-G2-}\alpha\text{-CNP} \xrightarrow{\alpha\text{-Amylase-Ca}^{2+}} \text{Gal-G2} + \text{CNP}$$

tCO_2

$$HCO_3^- + \text{PEP} \xrightarrow{\text{PEPC}} \text{oxaloacetate} + \text{phosphate}$$

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

3. Reagents

Included:

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

Reagent Composition:

Composition	Quantity/Panel
1,4-Piperazinediethanesulfonic Acid	0.08 mg
4-AAP	0.02 mg
ADP	0.03 mg
Arsenazo Ⅲ	7 μg
Bromocresol Green	5.4 μg
Creatinase	2.8 U
Creatininase	5.6 U
EDTA-calcium	0.4 mg
G6PDH	0.1 U
Gal-G2-α-CNP	0.1 mg
Glutamate Dehydrogenase	0.05 U
Lactate Dehydrogenase	0.6 U
Monosodium Phosphoenolpyruvate	0.062 mg
NAD	0.06 mg
Malate Dehydrogenase	0.055 U
phosphoenolpyruvate carboxylase	0.009 U
NADH	0.089 mg
ONPG	0.04 mg
Peroxidase	0.1 U

Composition	Quantity/Panel
Phosphoglucomutase	0.05 U
Pyruvate Kinase	0.05 U
Sarcosine Oxidase	0.4 U
Sucrose	0.3 mg
Sucrose Phosphorylase	0.01 U
TBHBA	0.2 mg
Urease	0.03 U
α-Amylase	0.2 U
α-Ketoglutaric Acid	0.05 mg
β-Galactosidase	0.3 U

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 Renal 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 Renal 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
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
CREA	Canine	0.4 - 1.6	mg/dL	35 - 141	μmol/L
	Feline	0.7 - 2.0	mg/dL	62 - 177	μmol/L
Ca	Canine	7.9 - 12.0	mg/dL	2.0 - 3.0	mmol/L
	Feline	8.0 - 12.0	mg/dL	2.0 - 3.0	mmol/L
PHOS	Canine	2.5 - 6.8	mg/dL	0.8 - 2.2	mmol/L
	Feline	3.1 - 7.5	mg/dL	1.0 - 2.4	mmol/L
Na	Canine	138 - 160	mmol/L	138 - 160	mmol/L
	Feline	142 - 164	mmol/L	142 - 164	mmol/L
K	Canine	3.5 - 5.8	mmol/L	3.5 - 5.8	mmol/L
	Feline	3.5 - 5.8	mmol/L	3.5 - 5.8	mmol/L

Test Item		Reference Interval		Reference Interval (SI Unit)	
Cl	Canine	106 - 120	mmol/L	106 - 120	mmol/L
	Feline	112 - 126	mmol/L	112 - 126	mmol/L
tCO_2	Canine	12 - 27	mmol/L	12-27	mmol/L
	Feline	15 - 24	mmol/L	15 - 24	mmol/L

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%
BUN	500 mg/dL	42.1 mg/dL	29.3 mg/dL	0.43%
CREA	200 mg/dL	25.9 mg/dL	---	0.17%
Ca	600 mg/dL	56.3 mg/dL	57.5 mg/dL	0.3%
PHOS	500 mg/dL	42.1 mg/dL	57.5 mg/dL	0.13%
Na	600 mg/dL	40.2 mg/dL	39.8 mg/dL	0.2%
K	100 mg/dL	40.2 mg/dL	22.8 mg/dL	0.15%
Cl	300 mg/dL	47.1 mg/dL	44.9 mg/dL	0.4%
tCO_2	530mg/dL	41.5 mg/dL	42.4mg/dL	0.16%

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
BUN	2.0 - 140	mg/dL	0.7 - 50.0	mmol urea/L
CREA	0.3 - 20.0	mg/dL	27 - 1768	μmol/L
Ca	4.0 - 15.0	mg/dL	1.0 - 3.8	mmol/L
PHOS	0.4 - 18.0	mg/dL	0.2 - 6.5	mmol/L
Na	110 - 175	mmol/L	110 - 175	mmol/L
K	1.5 - 8.5	mmol/L	1.5 - 8.5	mmol/L
Cl	70 - 140	mmol/L	70 - 140	mmol/L
tCO_2	10 - 40	mmol/L	10 - 40	mmol/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
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
CREA	Canine	0.9968	1.0526	-0.0305	38	0.5-16.9 mg/dL
	Feline	0.9928	1.0498	-0.2650	38	1.0-17.7 mg/dL
Ca	Canine	0.9888	1.0000	0.0000	38	7.3-16.4 mg/dL
	Feline	0.9823	0.9966	0.2615	34	6.3-14.1 mg/dL
PHOS	Canine	0.9855	1.0469	-0.5006	23	2.3-13.5 mg/dL
	Feline	0.9862	1.0223	-0.2665	22	4.5-12.2 mg/dL
Na	Canine	0.9854	0.9969	0.7604	40	116-178 mmol/L
	Feline	0.9863	0.9887	1.5809	47	125-175 mmol/L
K	Canine	0.9805	0.9728	0.1666	33	3.9-7.7 mmol/L
	Feline	0.981	1.0343	-0.1891	47	2.3-7.2 mmol/L
Cl	Canine	0.9804	0.9902	1.30159	36	93-136 mmol/L
	Feline	0.9819	0.9802	2.4583	28	90-146 mmol/L
tCO_2	Canine	0.9846	0.9218	2.7611	18	19.2-41.8 mmol/L
	Feline	0.9802	1.0766	-2.3002	17	13.1-36.7 mmol/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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Issue Date: 2012/03/19

Revised Date: 2020/08/21

PN:7B25000030HJ