

CLINICAL CHEMISTRY SCREEN

PRESENTATION

The clinical chemistry screen consists of automated procedures enabling the high-throughput screening of large numbers of mice for a broad range of clinical chemical parameters in blood and urine including substrates, electrolytes and enzymes, endocrine and metabolic parameters, as well as hematological / coagulation parameters.

A large panel of blood and urine analyses is available. The parameters selected allow to explore various organ systems (liver, kidney, cardiovascular...) and to detect changes in metabolic pathways and electrolytes homeostasis. Assays also evaluate blood hematology, coagulation and immunology parameters. In addition, most of the endocrine functions can be evaluated through the measurement of several circulating hormones.

While a list of parameters have been selected in primary phenotyping package flow scheme, alternative or additional individual analysis can be done as required.





1. BLOOD CHEMISTRY

Primary screen plasma chemistry:

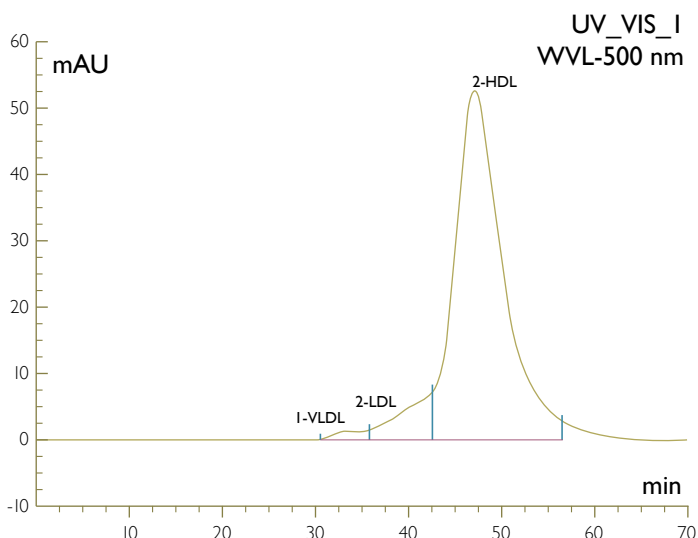
- Ions and metabolites: Sodium, Potassium, Chloride, glucose, urea, creatinin, uric acid, total proteins, albumin, calcium, inorganic phosphorus, iron, total cholesterol, triglycerides.
- Enzymatic activities: Alpha-amylase, aspartate amino transaminase (AST), alanine amino transaminase (ALT), creatine kinase (CK), alkaline phosphatase (ALP), lactate dehydrogenase (LDH).

Other parameters:

Ferritin, transferrin, total bilirubin, bile acids (total and profiling of specific acids), magnesium, bicarbonates, fructosamine, ketone bodies (beta hydroxybutyrate), glycerol.

Analysis of plasma lipids and lipoproteins:

- Plasma or serum triglycerides, glycerol, total, LDL and HDL cholesterol, free fatty acids.
- Lipoprotein profile by FPLC.



No.	Ret. Time	Peack Name	Height	Area	Rel. Area	Amount	Type
	min		mAU	mAU * min	%		
1	33.24	VLDL	2.042	8.561	2.36	0.064	M *
2	38.94	LDL	4.488	31.282	8.61	0.233	M *
3	47.13	HDL	53.560	323.632	89.04	2.413	M *
Total			60.090	363.476	100.00	2.710	M *

Cholesterol profile in C57BL/6j mouse plasma

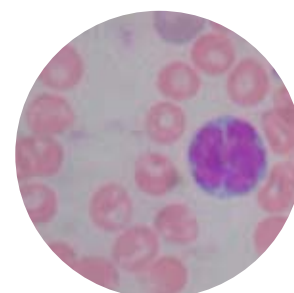
2. HEMATOLOGY

Basic hematology test

It includes determination of white and red blood cell counts, hematocrit, hemoglobin, erythrocyte indexes (Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration, Mean Corpuscular Volume), and platelets.

Differential blood counts

Neutrophils (segmented, not-segmented), lymphocytes, monocytes, eosinophils, basophils, presence of precursor cells (e.g. blasts, pro-myelocytes, myelocytes, meta-myelocytes), and changes in red blood cells number and morphology are determined by microscopic analysis



Erythropoietin (EPO) measurement

3. COAGULATION

A first line panel of analysis allows screening of the global status of coagulation: Prothrombine Time, Activated Partial Thromboplastin Time and fibrinogen.

4. IMMUNOLOGY

Plasma immunoglobulins

IgG1, IgG2a, IgG2b, IgG3, IgM, IgA and IgE.

FACS analysis

- Peripheral blood*: Total T-cell, T-cell CD4, T-cell CD8, Treg-cell CD25, B-cell-CD19, IgM/IgD, NonB/NonT (CD3-, CD19-), Monocyte-F4/80, Granulocyte Gr1

- Analysis of thymus and spleen populations:

Thymus:

T cells of the αβ lineage: CD4/CD8/TCRαβ

T cells of the γδ lineage: CD4/CD8/TCRγδ

NKT cells: NK1.1/CD3

Spleen:

B cells: CD19/IgM/IgD/CD80

B cells: CD19/IgM/CD21/CD23

NK (natural killer) cells and NKT cells: DX5/NK1.1/CD3

Macrophages and neutrophils: F4-80/Gr-1/Mac-1

T cells: CD4/CD8/CD3/CD44

Cytokines

A panel of 20 mice cytokines can be measured in plasma or serum by Multiplex analysis (IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, TNF-α, GM-CSF, G-CSF, IFNγ, IP-10, KC, MCP-1, MIP-1α, RANTES)*

Autoimmune antibodies*

- Anti-nuclear antibodies
- Anti-DNA antibodies
- Rheumatoid factor
- Anti-thyroglobulin antibodies



5. ENDOCRINOLOGY

I- The measurement of different **endocrine panels using the Multiplex technology** has been developed. This technology allows measuring simultaneously about 20 different parameters on 5-50 μl sample.

- Mouse endocrine Panel:

This multiplex assay kit enables the simultaneous quantitative determination of the following five mouse endocrine hormones in any combination: Insulin, Amylin*, Leptin, Glucagon*, and GLP-1*

- Mouse Adipokine

This multiplex assay kit is used to measure Leptin, Resistin, IL-6, TNFα, MCP-1, PAI-I and Insulin in mouse serum or plasma.

- Mouse gut hormones

This Mouse Gut Hormone multiplex assay simultaneously measures the hormones GLP-1* (active), GIP* (total), Ghrelin* (active), PYY* (total), PP*, Amylin* (active), Leptin, and Insulin. This panel is a useful tool for studying the biological functions of these hormones in the gut and in determining the physiological implications that these peptide hormones may have throughout the body.

- Mouse Adiponectin

This multiplex assay kit is used to measure Adiponectin in serum and plasma samples. Adipocyte extracts and culture media samples could also be assayed with this kit.

- Cardiovascular panels:

- Mouse CVD1

This multiplex assay kit is used to measure MMP-9*, PAI-1 (total)*, sE-selectin*, SICAM-1*, and sVCAM-1* in mouse serum or plasma.

- Mouse CVD2

This multiplex assay kit is used to measure Adiponectin, Apo-A1*, ApoE* and Fibrinogen* in mouse serum or plasma.

- Thyroid hormones:

This multiplex assay kit is used to measure T3 and T4 in mouse serum or plasma.

- Bone metabolism:

3 different panels of tests allow to explore bone metabolism including Osteocalcin*, OPG*, ACTH*, leptin, TNF- α *,.....

2- Steroid hormones and **growth hormones** are measured by classical RIA or ELISA technology: Corticosterone, estradiol and progesterone GH, IGF1

3- Renin Angiotensin*

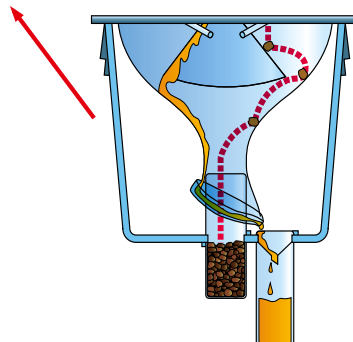
4- Pro atrionatriuretic peptide (Pro ANP)

6. URINE CHEMISTRY / RENAL FUNCTION

- Urine produced during 24h is collected in metabolic cages (Tecniplast). Urine chemistry including urine sodium, potassium, urea, creatinin, calcium, phosphorus, total proteins and glucose is performed. Urine osmolarity is analysed by using a Fiske 110 osmometer. Creatinine clearance and fractional sodium reabsorption are calculated. Urine albumin* is performed by ELISA.

- Urinary protein excretion:

Qualitative analysis of urinary protein is performed by SDS-Page electrophoresis



* validation in progress