

Workshop

كلية العلوم
وحدة تطوير المهارات وخدمة المجتمع

The reflatron apparatus and its physiological and biological applications of living organism

The Reflatron

جهاز الريفلوترون و تطبيقاته الفسيولوجية و الحيوية للكائن الحي

Sunday 20/1/02019 - 14/5/1440

By

Dr. Dalia Fouad

Associate professor

Department of zoology; college of science

King Saud University

Workshop

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The reflatron apparatus and its physiological and biological applications of living organism

فريق العمل

د/داليا فؤاد محمد استاذ مشارك

ا/ جواهر الغامدى معيدة

ا/ امل المهنا معيدة

ا/ ريم المقحم فنية مختبر

Reflotron® Plus system



Flexible testing to support your clinical decisions

- The Reflotron Plus system is a single-test clinical chemistry system which allows the measurement of 17 parameters from whole blood, plasma or serum – including liver and pancreas enzymes, metabolites, blood lipids, hemoglobin and potassium. Immediate and reliable test results ensure quick performance and verification of the diagnosis without delay. The system is suitable for primary care settings, as a back-up system in hospitals and private labs, at screening sites and for health check-ups.



- **Reliability**
- Excellent test strip stability minimizing storage concerns
- Little waste and almost no maintenance
- **Faster clinical decision making**
- Quick time to result
- No reagent preparation
- **Specifications**
- Throughput of Reflotron® Sprint: up to approx. 60 tests/hour
- Throughput of Reflotron Plus: up to approx. 25 tests/hour
- **Sample material:** whole blood (capillary and venous) plasma or serum
- **Sample volume:** 30 µL
- **Time-to-result:** only 2 – 3 min. (depends on parameter)
- **Integrated printer:** immediate documentation of results
- Barcode reader and/or keyboard for patient and sample ID input



Muscle diseases



Anemia



Lipid metabolism disorders



Bone diseases



Liver diseases



Renal diseases



Gout



Diabetes / Pancreatitis



Reflotron® Tests and Indications

Acute Parameters

- Potassium
- CK
- Haemoglobin
- Glucose
- Creatinine
- α-Amylase
- Pancreatic Amylase
- AST/GOT

Kidney diseases

- Urea
- Creatinine
- Potassium
- Uric Acid
- Haemoglobin

Diabetes

- Glucose
- Triglycerides
- HDL Cholesterol
- Creatinine

Pancreatitis

- Pancreatic Amylase
- Amylase

Liver diseases

- AST/GOT
- ALT/GPT
- γ-GT
- Bilirubin
- Alkaline Phosphatase

Anaemia

- Haemoglobin
- Bilirubin

Muscle disease (myocardial infarction)

- CK

Lipid disorders

- Cholesterol
- Triglycerides
- HDL Cholesterol
- LDL Cholesterol
- Glucose

Gout

- Uric Acid
- Urea
- Creatinine
- Glucose
- Cholesterol
- Triglycerides

Functions Of The reagent strip

1. Plasma separation and pre-incubation
2. Plasma transport
3. Reaction and dye formation Underside of the reagent strip
4. Transfer of test- and lot-specific information to the photometer (magnetic stripe)

Reflotron® reagent strip

- The following illustration depicts a typical Reflotron® reagent strip.

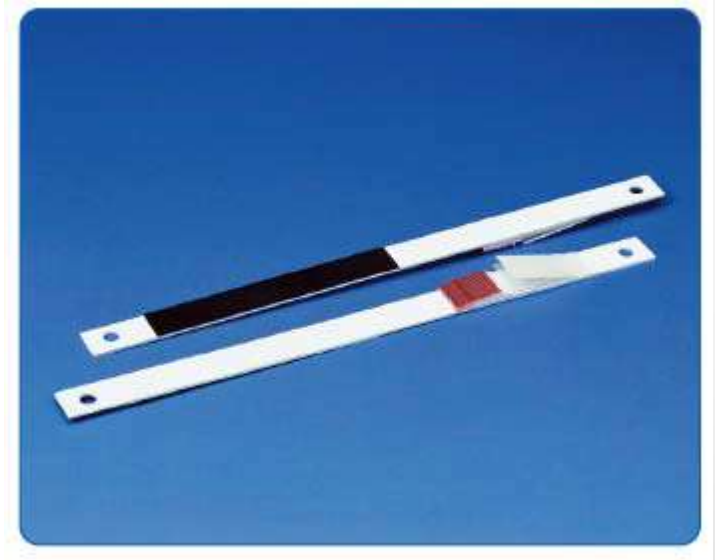


Figure 1
Reagent strip for Reflotron®;
top and underside

Reflotron® reagent strip

Figure 2 is a schematic representation of the principal components of a Reflotron® reagent strip. On the upper side of the carrier strip is the test zone consisting of several discrete areas in which various processes and reactions take place.

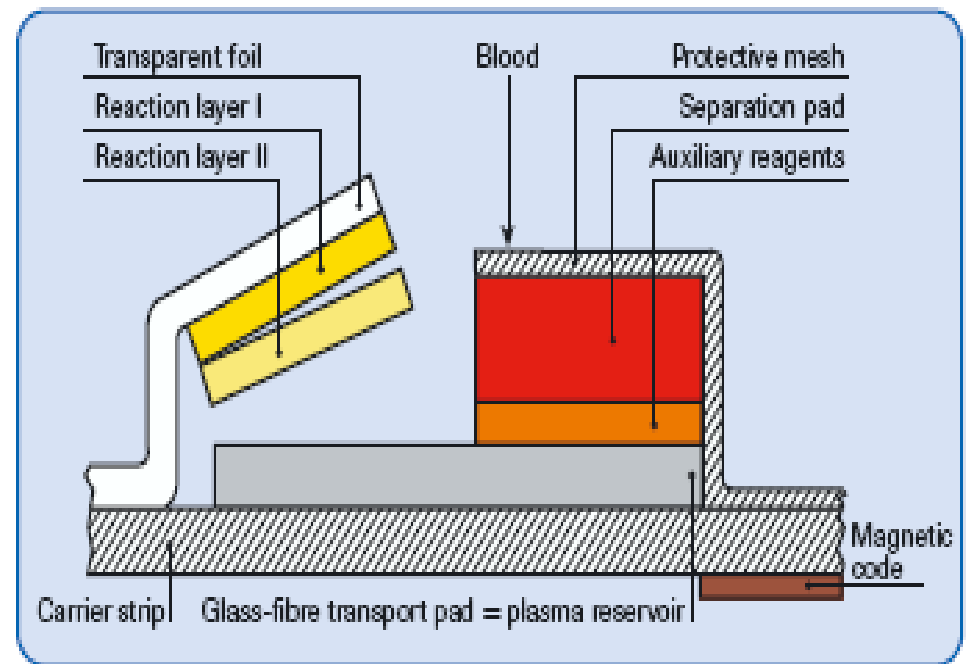


Figure 2
Schematic diagram of a reagent strip

Integrated plasma collection

- A Reflotron® or capillary pipette is used to apply 30 μL of sample (blood, serum or plasma) to the red mesh covering the separation pad. A yellow mesh is used for potassium and HDL cholesterol to indicate that serum or plasma is required as sample material.
- The sample passes through the separation pad which, when blood is the sample material, separates out erythrocytes and other cellular components. The plasma or serum that is obtained passes (sometimes through an additional pre-reaction zone) to the transport pad and hence underneath the reaction zone.
- Having arrived there the sample (more than is actually required) is available for the test reaction. The photometer starts the analyte determination reaction at a defined time by pushing the reaction zone onto the transport pad. The reaction layers absorb from the transport pad by capillary action the quantity of plasma or serum required for the reaction (Figure 3).



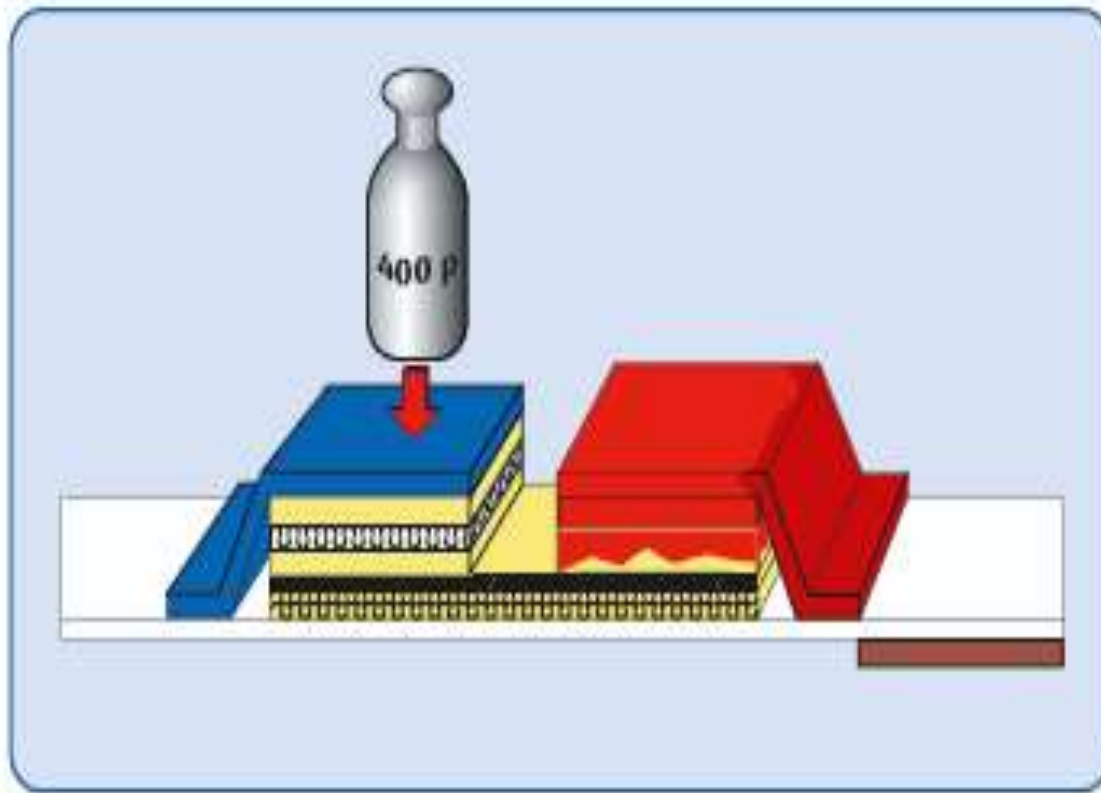


Figure 3
Schematic diagram of
automatic sample
dosing

This principle of automatic dosing by the reaction layers enables always the same volume of sample to be applied for blood, plasma or serum.

An exception to this is the Reflotron® Hemoglobin reagent strip. Instead of the glass-fibre pad it incorporates a material that has been saturated with saponin to induce erythrocyte haemolysis and also contains the reactive components for converting haemoglobin to methaemoglobin.

Table 2: Reflotron® Tests: test principles

Test

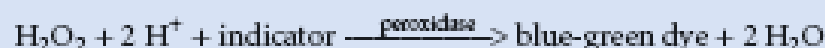
Test principle

Wavelength

Glucose



642 nm



Indicator: 3,3',5,5'-tetramethylbenzidine

Cholesterol



642 nm



Indicator: 3,3',5,5'-tetramethylbenzidine

HDL cholesterol

1. Precipitation of chylomicrons, VLDL and LDL with dextran sulphate/Mg²⁺

642 nm

2. Determination of HDL cholesterol



Indicator: 4-(4-dimethylaminophenyl)-5-methyl-2-(3,5-dimethoxy-4-hydroxyphenyl)imidazole dihydrochloride

Test

Test principle

Wavelength

Triglycerides

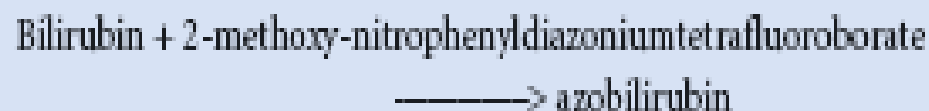


642 nm



Indicator: 4-(4-dimethylaminophenyl)-5-methyl-2-(3,5-dimethoxy-4-hydroxyphenyl)imidazole dihydrochloride

Bilirubin

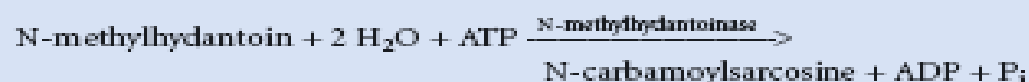


567 nm

Indirect bilirubin is released by means of dyphilline

Test principle

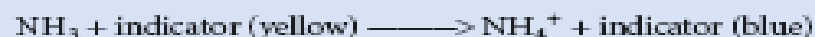
Wavelength

Creatinine

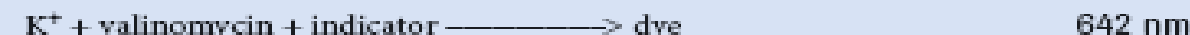
Indicator: 2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-4-(5)-(9-julolidino)-5-(4)-methyl-(1H)-imidazole

Haemoglobin**Uric acid**

Indicator: 4-(4-dimethylaminophenyl)-5-methyl-2-(3,5-dimethoxy-4-hydroxyphenyl)imidazole dihydrochloride

Urea

Indicator: tetrachlorophenoltetrabromosulphophthalein

Potassium

Indicator: 4-[(2,6-dibromo-4-nitrophenyl)azo]-2-octadecyloxy-1-naphthol; 2,4,6,8-tetranitro-S-octadecyloxy-1-naphthol

Test principle

Wavelength

Alkaline Phosphatase

α -cresolphthalein phosphate + methylglucamine
 $\xrightarrow{\text{ALP}}$ α -cresolphthalein + methylglucamine phosphat

567 nm

Amylase

indolyl- α , D-maltoheptaoside $\xrightarrow{\alpha\text{-amylase}/\alpha\text{-glucosidase}}$ indoxyl + glucose
 indoxyl + 2-methoxy-4-morpholinophenyldiazoniumtetrachlorozinkate
 \longrightarrow purple dye

567 nm

Pancreatic Amylase

1. Inhibition of salivary amylase with monoclonal antibodies
 indolyl- α , D-maltoheptaoside $\xrightarrow{\alpha\text{-amylase}/\alpha\text{-glucosidase}}$ indoxyl + glucose
 indoxyl + 2-methoxy-4-morpholinophenyldiazoniumtetrachlorozinkate
 \longrightarrow purple dye

567 nm

CK

creatine phosphate + ADP $\xrightarrow{\text{CK}}$ creatine + ATP
 glycerol + ATP $\xrightarrow{\text{glycerol kinase}}$ glycerol-3-phosphate + ADP
 glycerol-3-phosphate + O₂ $\xrightarrow{\text{glycerol phosphate oxidase}}$ dihydroxyacetone phosphate + H₂O₂
 H₂O₂ + indicator $\xrightarrow{\text{peroxidase}}$ indicator (ox.) + H₂O
 Indicator: 2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-4-(5)-(9-julolidino)-5-(4)-methyl-(1H)-imidazole

642 nm

γ -GT

glycylglycin + γ -glutamyl-3-carboxy-1,4-phenylene diamine
 $\xrightarrow{\gamma\text{-GT}}$ γ -glutamylglycyl-glycine + 3-carboxy-1,4-phenylene diamine
 3-carboxy-1,4-phenylene diamine + N-methylantranilic acid + 6 [Fe(CN)₆]³⁻
 \longrightarrow dye + 6 [Fe(CN)₆]⁴⁻

642 nm

GOT (AST)

α -ketoglutarate + alanine sulphinate $\xrightarrow{\text{GOT}}$ glutamate + pyruvate + SO₃²⁻
 pyruvate + PO₄³⁻ + O₂ + H₂O $\xrightarrow{\text{pyruvate oxidase}}$ acetylphosphate + H₂O₂ + CO₂
 H₂O₂ + indicator (red.) $\xrightarrow{\text{peroxidase}}$ indicator (ox.) + H₂O
 Indicator: 4-(4-dimethylaminophenyl)-5-methyl-2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)imidazole dihydrochloride

567 nm

GPT (ALT)

α -ketoglutarate + alanine $\xrightarrow{\text{GPT}}$ glutamate + pyruvate
 pyruvate + PO₄³⁻ + O₂ + H₂O $\xrightarrow{\text{pyruvate oxidase}}$ acetylphosphate + H₂O₂ + CO₂
 H₂O₂ + indicator (red.) $\xrightarrow{\text{peroxidase}}$ indicator (ox.) + H₂O
 Indicator: 4-(4-dimethylaminophenyl)-5-methyl-2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)imidazole dihydrochloride

567 nm

Transfer of test- and lot-specific information

- **Magnetic Strip:**

On the underside of the Reflotron[®] reagent strip is a magnetic strip that is encoded with information relating to the test and to that particular lot of strips. The photometer reads the stripe immediately after the strip has been inserted. The strip contains all the information it needs to carry out the test:

Test parameter

- **Time/process settings**

- o Plasma separation time
- o Rate at which the Ulbricht's sphere advances
- o Ventilation time
- o Reaction time
- o Number of measurements
- o Kinetic measurement
- o End-point measurement

- **LED configuration**

- o Measuring wavelength
- o Calculation factors

- **Evaluation constants**

- o Conversion factors for units of measurement (Con/SI)
- o Conversion factors for temperature (37°C; 30°C; 25°C)
- o Measuring range limits

- **Linearity limit values for enzyme determinations**

Transfer of test- and lot-specific information

- The photometer uses a checkcode to verify that it has correctly read the data from the magnetic strip. While most of the data remain constant for a given parameter, the characteristic data that are used to calculate the result from the reflectance readings are determined anew for each lot of reagent strips. This balances out the inevitable lot-to-lot differences that occur during production as a result of changing from one lot of raw material to another.
- Calibration by the user is therefore unnecessary.

Testing procedure

- The Reflotron® system, unlike other clinical-chemical systems, does not require calibration by the user, as the manufacturer calibrates the reagent strips lot by lot and encodes the data on the reagent strip, which the instrument reads.
- The test strip is designed to make testing quick and simple. The silver foil is removed from the test strip and sample is added directly to the test strip.



of
so be

Testing procedure

- **Just potassium and HDL cholesterol** require the use of serum or plasma. **Serum or plasma** may be used for the other tests, too, of course, with the exception of the haemoglobin test.
- Sample is applied to the reagent strip with an ordinary capillary or laboratory pipette, the reagent strip is placed in the photometer and testing is started by closing the measuring chamber flap.
- **Fast test result:**
- The time to result is approximately 2-3 minutes. Lipid results can be used directly for other calculations such as LDL cholesterol, myocardial infarction risk, and the cholesterol/HDL cholesterol quotient. If desired, creatinine values can be used to measure creatinine clearance. The formula for performing the calculation is programmed into the photometer.

Measuring procedure

- **Test reaction:**
- Closing the measuring chamber flap moves the strip to the measuring position and automatically starts the measuring procedure. The photometer first reads, checks and saves the data encoded on the magnetic stripe on the underside of the reagent strip. Strip recognition is then complete and the instrument carries out the measurement strictly according to the data it has received from the strip.
- Before the reaction starts, erythrocytes are separated out of the blood sample. During the incubation phase, the plasma reservoir fills and the photometer heats the reagent strip to 37°C.
- The reaction commences when the reactive layers are pressed by the measuring head, an Ulbricht's sphere, into the plasma reservoir so bringing the reagents into

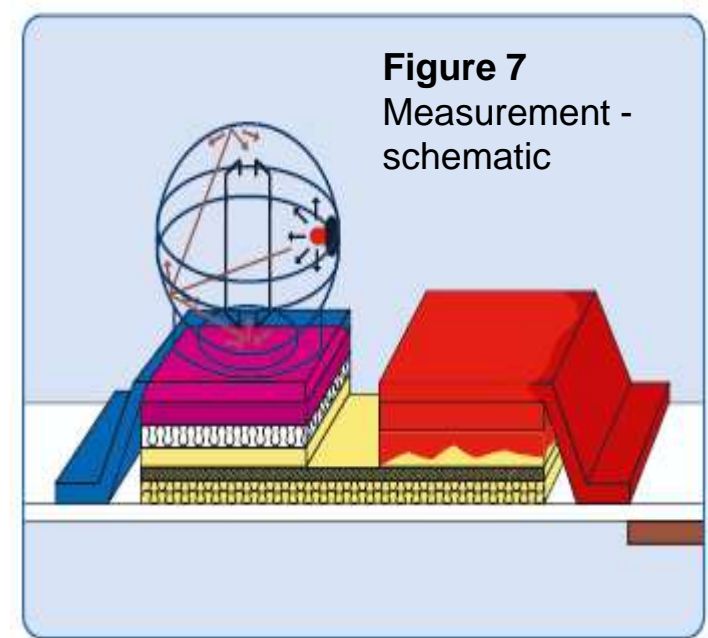


Figure 7
Measurement -
schematic

Sample can now enter the layers carrying the reagents. The intensity of colour of the reaction product is measured at various times (kinetic measurement) or on completion of the reaction (end-point measurement) (Figure 7).

Automatic calibration

- Sample can now enter the layers carrying the reagents. The intensity of colour of the reaction product is measured at various times (kinetic measurement) or on completion of the reaction (end-point measurement) (Figure 7).

Calibration values

- All according to the particular test, between 6 and 18 calibrators are grouped by the factory into sets covering the required analyte concentration range; where necessary through addition of analyte in order to achieve the necessary concentration.
- These calibrators are the link between the standard method used in formal clinical chemistry analysis in the laboratory and the values obtained using the Reflotron® test. This means that, despite the different test principles utilized in the formal and the Reflotron® systems, the values obtained from human specimen material remain comparable.

Calibration values

- Calibration of the tests is carried out using internationally applied reference methods so that comparability with these laboratory methods is assured (Table 3).
- The reference method values for a calibrator set are determined in reference laboratories, and each calibrator thereby receives a value that relates to the standard method. These calibrators are then measured using fresh human specimen material in parallel by the reference method and on the Reflotron®. In this way concentrations can be matched with reflectance values as measured by the Reflotron®.

These human serum or plasma-based calibrators are kept at -20° C to -70° C in order to guarantee stability. The use of frozen samples has the advantage that stable specimen material is available over a prolonged period in order to guarantee and document the comparability of different reagent strip lots for the same parameter.

Test	Reference Method
Alkaline Phosphatase	ALP IFCC liquid
Amylase	α-Amylase liquid (IFCC)
Pancreatic Amylase	Pancreatic- α-Amylase liquid
Bilirubin	DPD method
Cholesterol	CHOD-PAP method
CK	CK liquid
Creatinine	Creatinine plus
γ-GT	γ-GT liquid (IFCC)
Glucose	Hexokinase method
GOT (AST)	IFCC (without pyridoxal phosphate activation)
GPT (ALT)	IFCC (without pyridoxal phosphate activation)
HDL Cholesterol	Homogeneous HDL cholesterol plus method
Haemoglobin	SLS Haemoglobin method
Uric acid	Uric acid plus
Urea	Kinetic UV test
Potassium	Flame photometry
Triglycerides	GPO-PAP method

Table 3: Reference methods for the Reflotron® Tests

Measuring ranges

- The measuring ranges cover the entire clinically relevant concentration or activity range. Table 4 is a summary of measuring ranges of the Reflotron® Tests.

Table 4
Measuring ranges for
Reflotron® Tests

Glucose	0.56 – 33.3 mmol/l
Cholesterol	2.59 – 12.9 mmol/l
HDL Cholesterol	0.26 – 2.59 mmol/l
Triglycerides	0.80 – 6.86 mmol/l
Creatinine	44.5 – 884 µmol/l
Urea	3.33 – 50 mmol/l
Uric acid	120 – 1190 µmol/l
Bilirubin	8.5 – 204 µmol/l
Haemoglobin	3.1 – 12.4 mmol/l
Potassium	2.0 – 12.0 mmol/l
Alkaline Phosphatase	20 – 1250 U/l (37°C)
GPT (ALT)	5.0 – 2000 U/l (37°C)
GOT (AST)	5.0 – 500 U/l (37°C)
γ-GT	5.0 – 3500 U/l (37°C)
CK	24.4 – 1400 U/l (37°C)
Amylase	29 – 860 U/l (37°C)
Pancreatic Amylase	14 – 850 U/l (37°C)

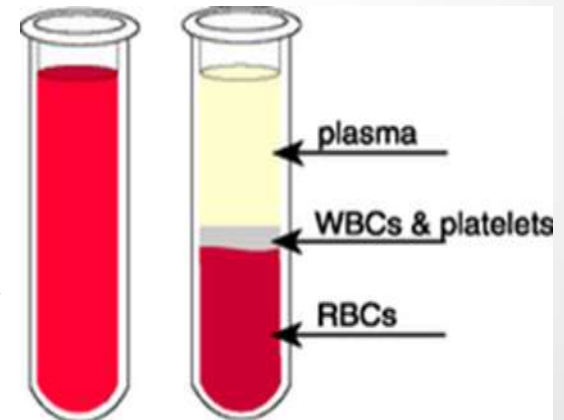
Blood collection

Blood makes up 6–8% of our total body weight.



Normal adult blood volume is 5 L.

Blood is made up of cellular material in a fluid called plasma.



- Blood is a circulating tissue consisting of three types of cells.

1. Red Blood Cells → Erythrocytes
2. White Blood Cells → Leukocytes
3. Platelets → Thrombocytes



- The cells listed above are suspended in a liquid known as plasma.

Each type of blood cell performs a different function.

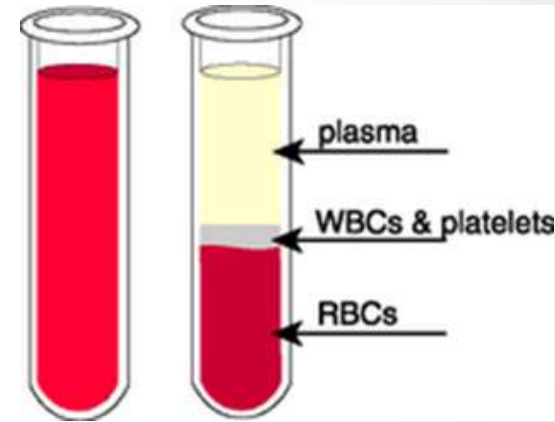
Red blood cells (Erythrocytes)














White blood cells (Leukocytes)

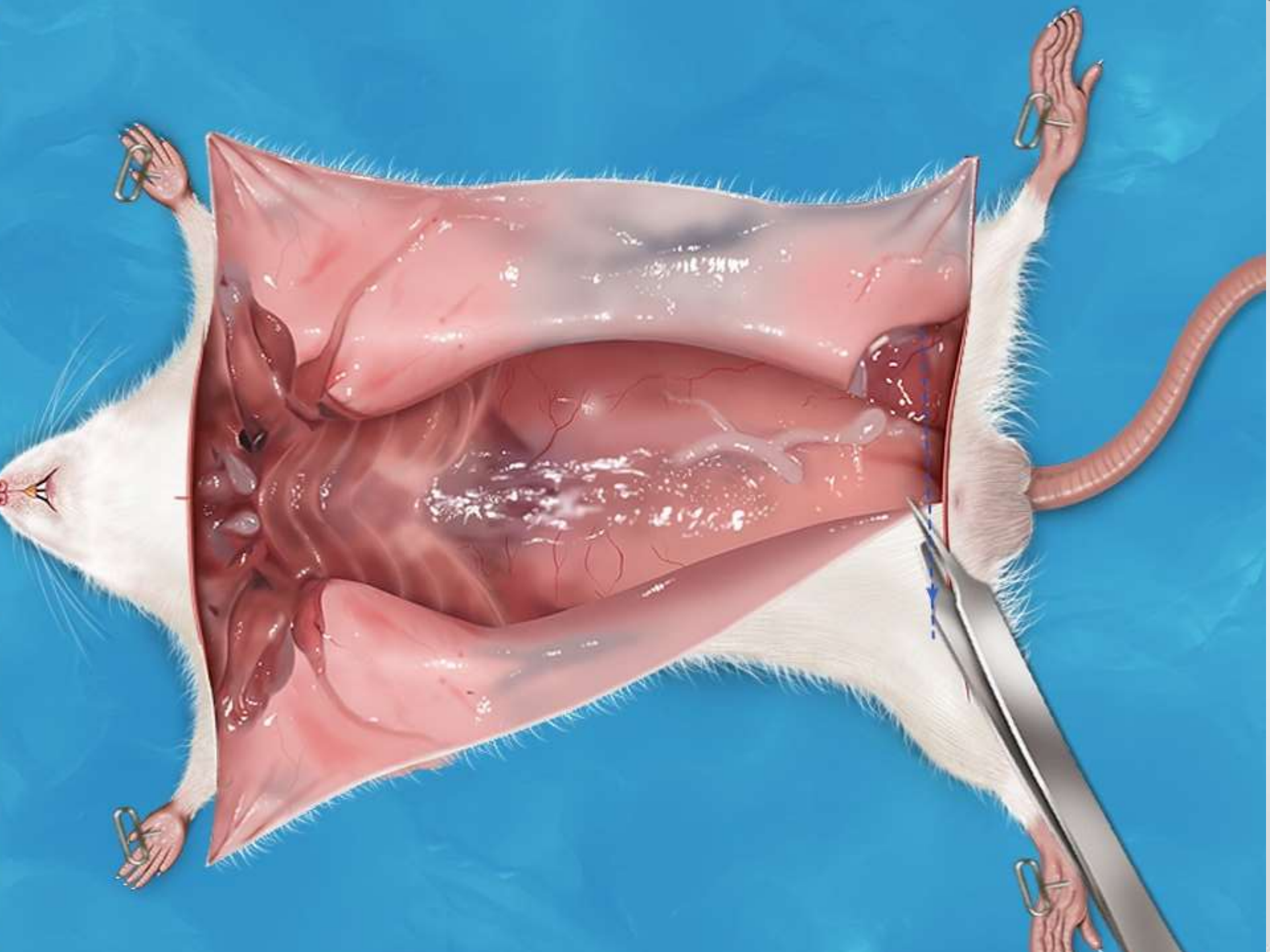


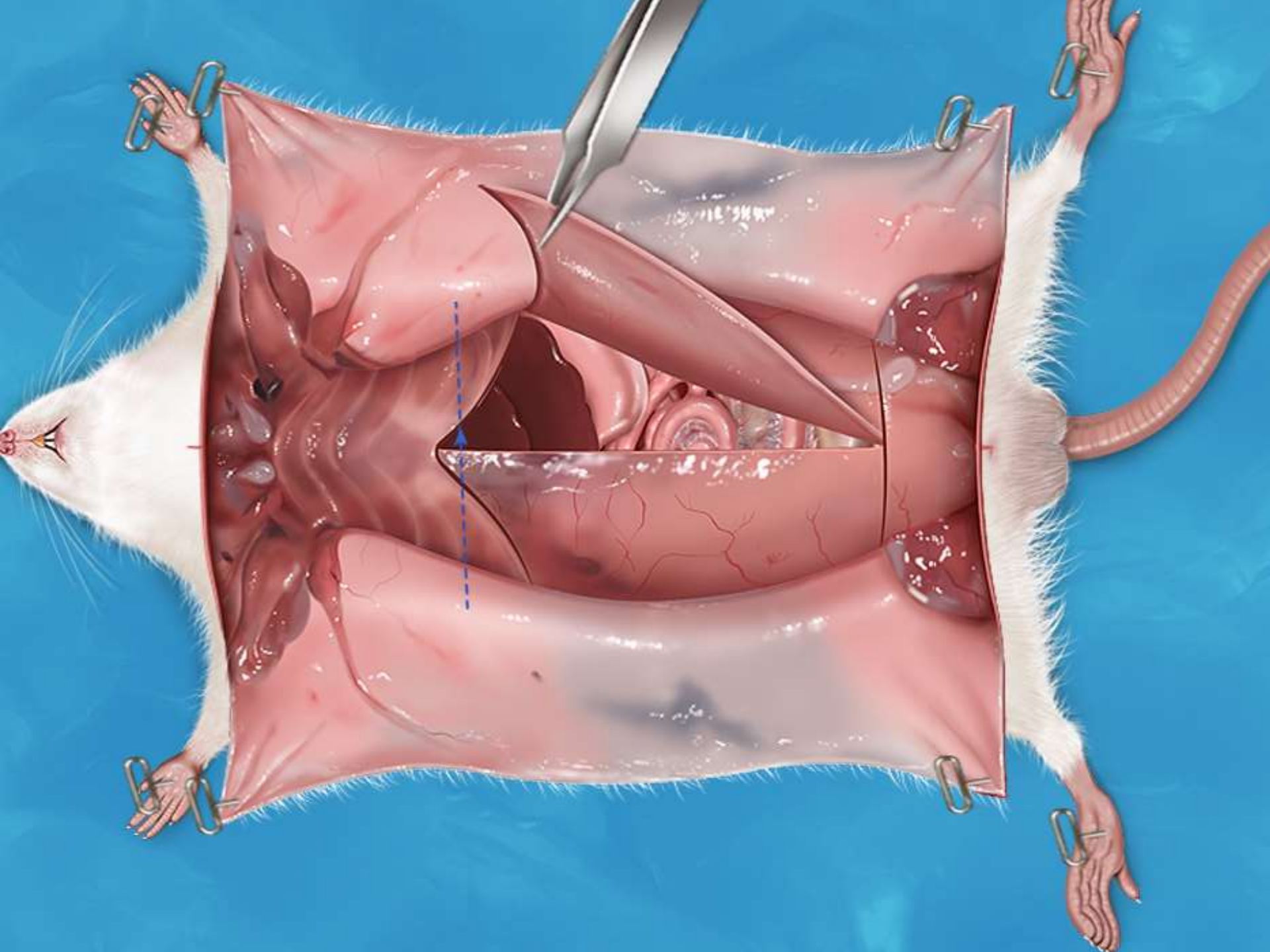
Platelets (Thrombocytes)



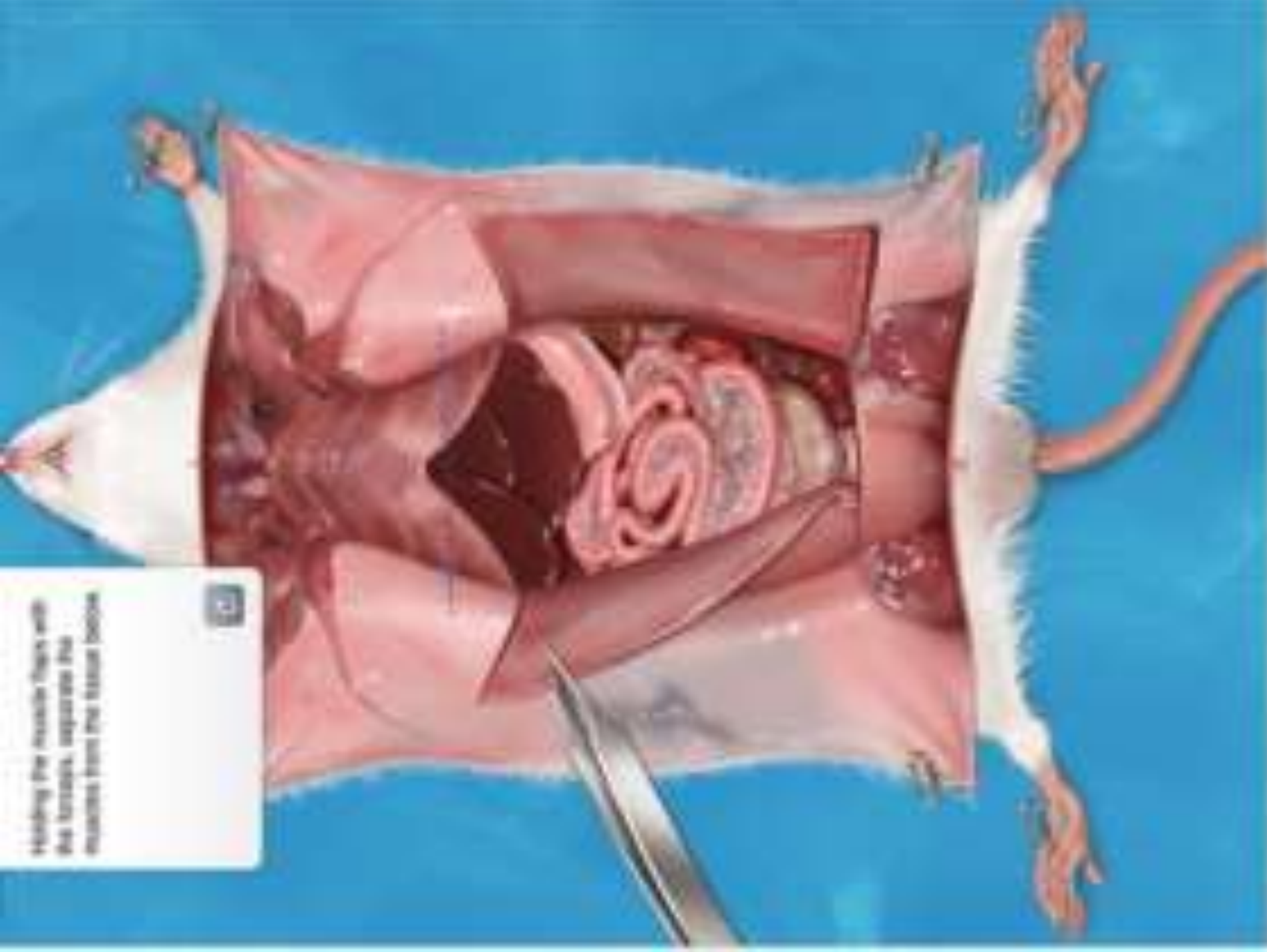
Tube Type and Order		Common Tests	Collection Instructions
	Blood Cultures	<u>Microbiology</u> : Septicemia, Toxemia and bacterial culture.	Aerobic (green) bottles MUST be collected first then Anaerobic (Orange). Pediatric Collection- Yellow bottle only. Mix the specimen with gentle agitation. Label bottles with Patient's full name, date of birth, date & time of collection and bar code. Store at Room Temperature.
	Sodium Citrate (BLUE)	<u>Hematology</u> : Prothrombin time, Coagulation Studies, INR, Factor VIII, Lupus Anticoagulant, D-Dimer, Protein C+S, APC, AT3, HPA100 (Platelet Function Test)	Note: Correct volume critical. See marker level on tube INVERT tube GENTLY 8-8 times after collection. Always label tubes with Patient's full name, date of birth, date & time of collection and bar code
	ACID (YELLOW)	<u>Basal Test</u> : HLA Tissue Typing, Flow Cytometry, Lymphocyte Surface Markers, TSB Cells, HLA-D27, EDTA (SUPPLE must accompany this tube)	Note: Correct volume critical INVERT tube GENTLY 8-8 times after collection Always label tubes with Patient's full name, date of birth, date & time of collection and bar code Collector MUST sign tubes
	Plasma (RED)	<u>Blood Bank</u> : Group and Hold, Cross Match <u>Biochemistry</u> : Therapeutic Drugs and Antibiotics, Serum Copper	INVERT tube GENTLY 8-8 times after collection Always label tubes with Patient's full name, date of birth, date & time of collection and bar code
	BIST (GOLD)	<u>Biochemistry</u> : Lipids, LFT's LVE, Creatinine, SUA, Cardiac Enzymes (Main Lab & Centaur sites only) Amplase, Calcium, Phosphate, Ionized Calcium, Protein Assays, TFF, pNOD (Main Lab & Centaur sites only) Iron Studies, C Reactive Protein, B 12, HbA, Insulin Endocrinology - Hormones <u>Serology/Immunology</u> : Hepatitis A, B, C, Allergy, Rubella, Rheumatoid Factor, EPQ, HIV, ANA, Viral antibodies, Syphilis	Collect extra tube for Hepatitis Serology or HIV Collect extra tube for Endocrinology Collect dedicated tube for Ionized Calcium- Seal and do not open prior to testing For Cholesterol Clearance : Collected tube when 24hr urine is collected. Always label tubes with Patient's full name, date of birth, date & time of collection and bar code
	Lithium Heparin (GREEN)	<u>Cytogenetics, Biochemistry</u> : Chromosomes, Red cell & Insulin - Organochlorines	INVERT tube GENTLY 8-8 times after collection Always label tubes with Patient's full name, date of birth, date & time of collection and bar code
	EDTA (PINK)	<u>Molecular Diagnostics</u> <u>Infectious PCR Testing</u> : Hep C PCR (Qual) / Hep C (RNA, HIV Viral Load, Hep C Genotype / Hep C (Quant) / Viral Load, Hep B (Quant) / Hep B Viral Load / Hep B DNA PCR. <u>Non-Infectious Genetic Testing</u> : Haemochromatosis gene test, Factor V Leiden genotyping, Prothrombin Gene Mutation, MTHFR Genotyping, JAK2 Mutation, MPL, Coeliac Gene testing, Alpha 1-Antitrypsin Gene testing, APO E	Infectious PCR Testing : Collect one tube/each. Mix well after collection by inverting GENTLY 8-8 times then Centrifuge, separate plasma and freeze. Non-Infectious Genetic Testing : Keep as whole blood and mix well after collection by inverting GENTLY 6-8 times. Refrigerate 17-20 C after collection. Always label tubes with Patient's full name, date of birth, date & time of collection and bar code
	EDTA (PURPLE)	<u>Hematology</u> : FBC, Blood Film, Hb, WCC, Diff, Platelets, Hb Electrophoresis (EPQ), Clotated Hb (HbA _{1c}), TSB Cells, ESR, Malaria Parasites (Thick & Thin Films), IM (Infectious Mononucleosis) <u>Biochemistry</u> : Red cell Folates, Carboxy-Hb, Manganese, Anionemia, Homocysteine, Troponin / Beta HCG (AQ130 - Regional and Perth peripheral labs) <u>Blood Bank</u> : Cross Match, Group and Hold, Blood Group Antibody Screen	INVERT tube GENTLY 8-8 times after collection Always label tubes with Patient's full name, date of birth, date & time of collection and bar code
	TRACE ELEMENT EDTA (DARK BLUE)	<u>Basal Chemistry</u> : Trace Metals including: Zinc, Lead, Cadmium, Aluminium, Selenium, Copper, Arsenic, Mercury, Cobalt, Chromium If 3 or more trace metals requested collect 2 tubes	INVERT tube GENTLY 8-8 times after collection Always label tubes with Patient's full name, date of birth, date & time of collection and bar code
	Fluoride Oxalate (GREY)	<u>Biochemistry</u> : Glucose, Alcohol, Lactate	INVERT tube GENTLY 8-8 times after collection Always label tubes with Patient's full name, date of birth, date & time of collection and bar code
	ESR (BLACK)	<u>Hematology</u> : ESR (Regional labs only)	Note: ESR tubes must be full draw volume. INVERT tube GENTLY 8-8 times after collection Always label tubes with Patient's full name, date of birth, date & time of collection and bar code

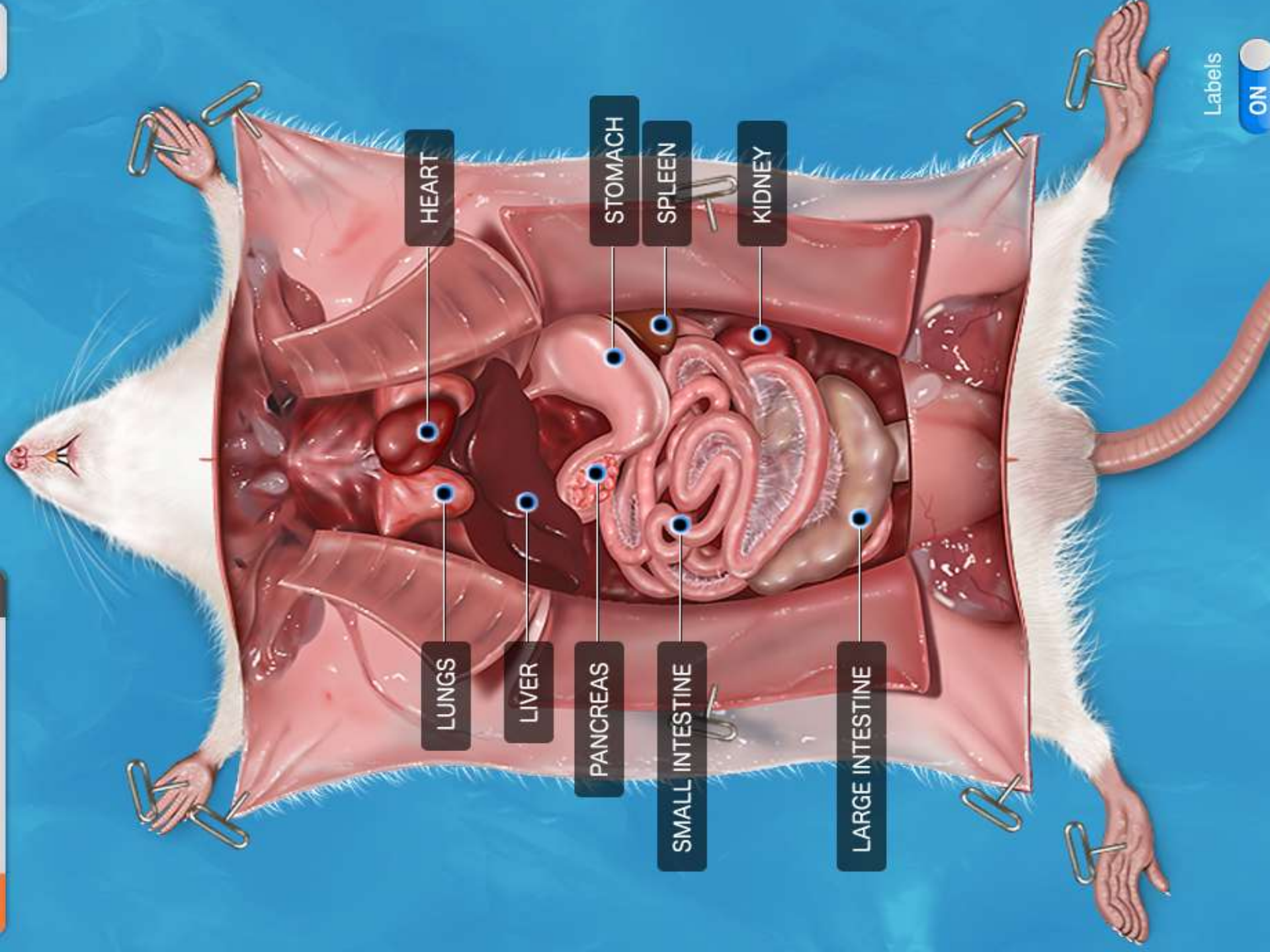
Rat Dissection





Holding the muscle flaps with the forceps, separate the muscles from the tissue below





HEART

LUNGS

LIVER

PANCREAS

STOMACH

SPLEEN

SMALL INTESTINE

KIDNEY

LARGE INTESTINE

Labels

ON

T. S. of The Liver

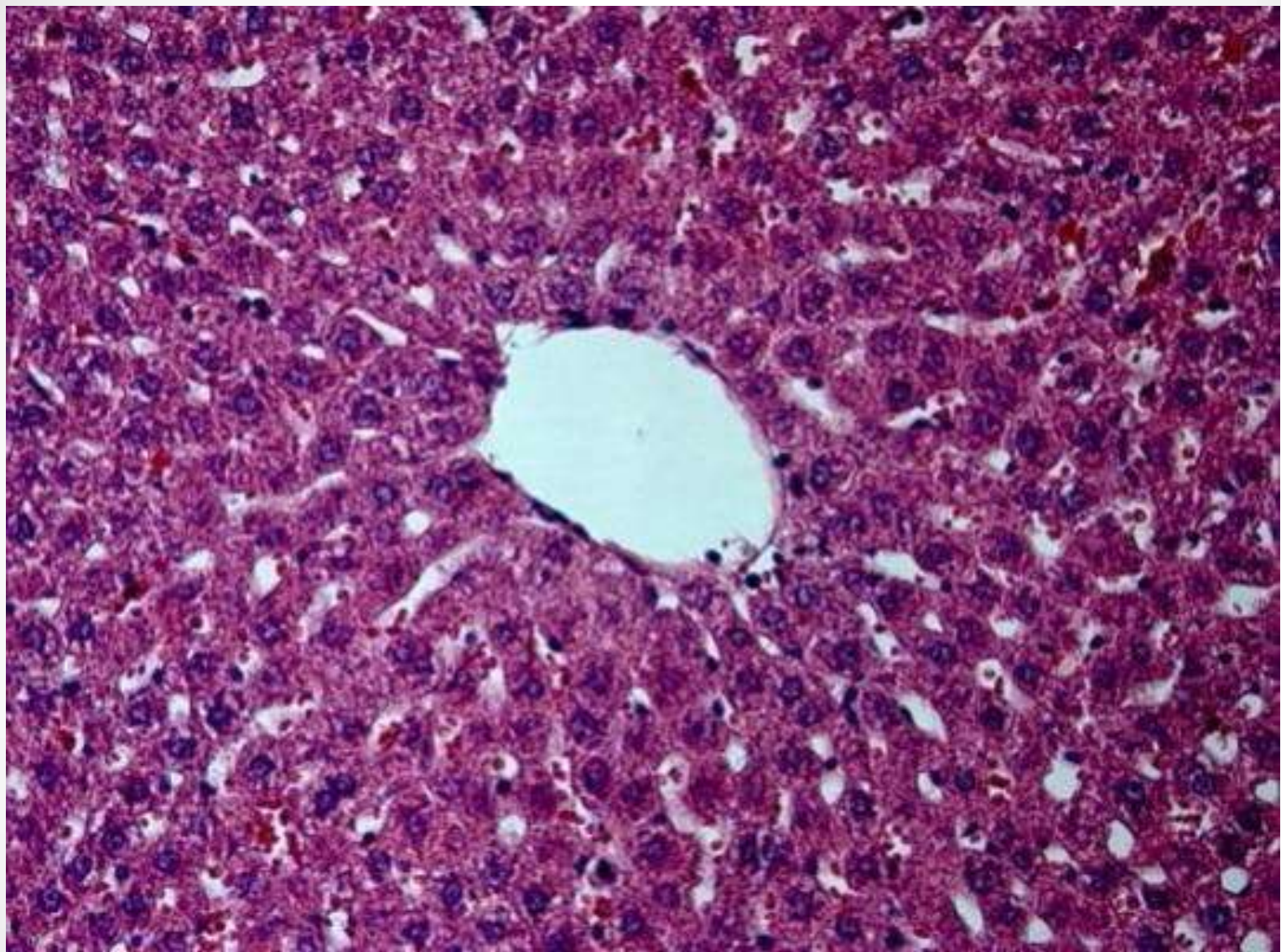


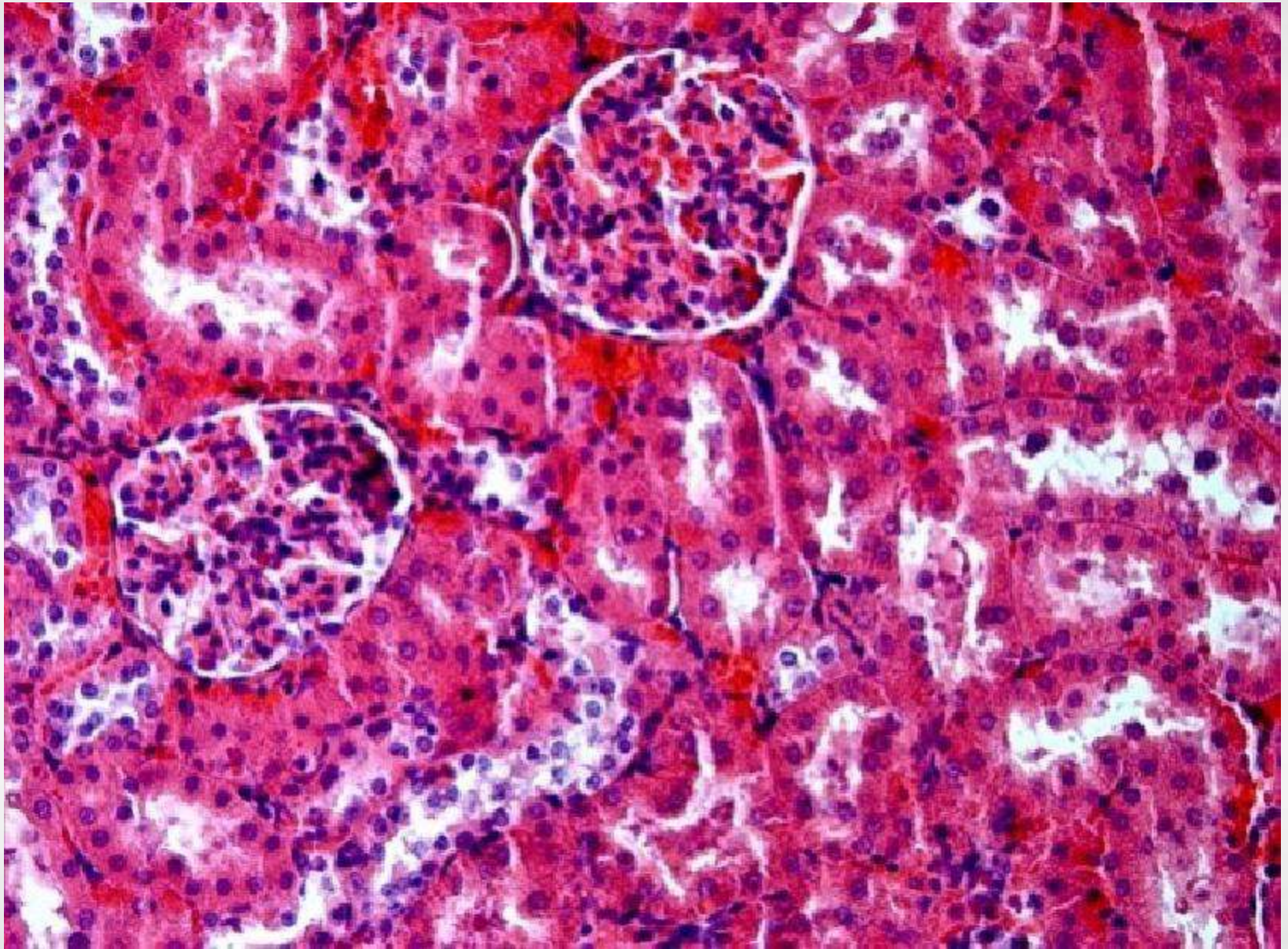
The portal area of The Liver

Branch of bile duct

Branch of hepatic portal artery

Branch of hepatic portal vein





Refranses

- <http://photos.labwrench.com/equipmentManuals/11023-6349.pdf>
- <https://www.roche.com/products/product-details.htm?productId=46ee3c74-7acf-4e96-9b3e-2277252dc207>
- <http://www.wdp.com.au/portals/0/WDP/WDP%20PathologyTube%20Chart%20and%20Order%20Of%20Draw%20v2%200.pdf>
- <https://www.commonsense.org/education/app/rat-dissection>
- <https://www.commonsense.org/education/app/rat-dissection>
- <http://www.ipadcurriculum.com/2011/06/virtual-rat-dissection-app/>

**Thank you for attention ..
Any question?**