Which samples for which uses?

Gauthier DOBIGNY & Philippe GAUTHIER – ObsMICE meeting, Niamey, dec. 2022

1- A short reminder:

Sampling procedures when dissecting

Cleaning of pathogens **and** nucleic acids

- ✓ Mechanic washing = water (best way to remove fluids, especially blood)

- ✓ Pathogens killed by heat, ethanol, javel, licensed desinfectants (e.g., phagospray, anios, etc)

  = desinfection

- ✓ Nucleic acids are not living organisms!

  RNA fragile, DNA robust (even at RT)

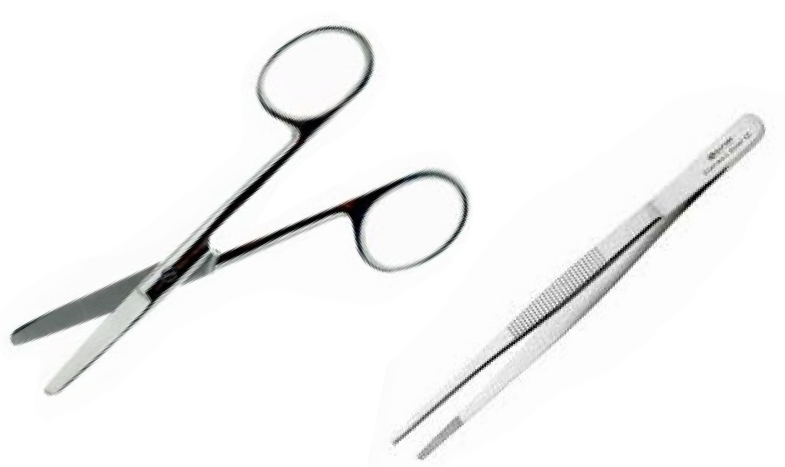
  Destroyed by specific (and expensive) solutions …

  or Sodium Hypochloride 10% (at least 10 minutes!) – WHO

  = decontamination

First pair to open the body

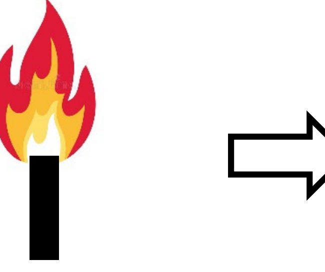
Second pair to sample viscers



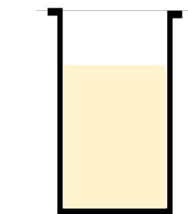
Dissection 1

Water
≠ blood

« Dirty » ethanol + flame
≠ pathogens and DNA/RNA



Javel
(to wait)



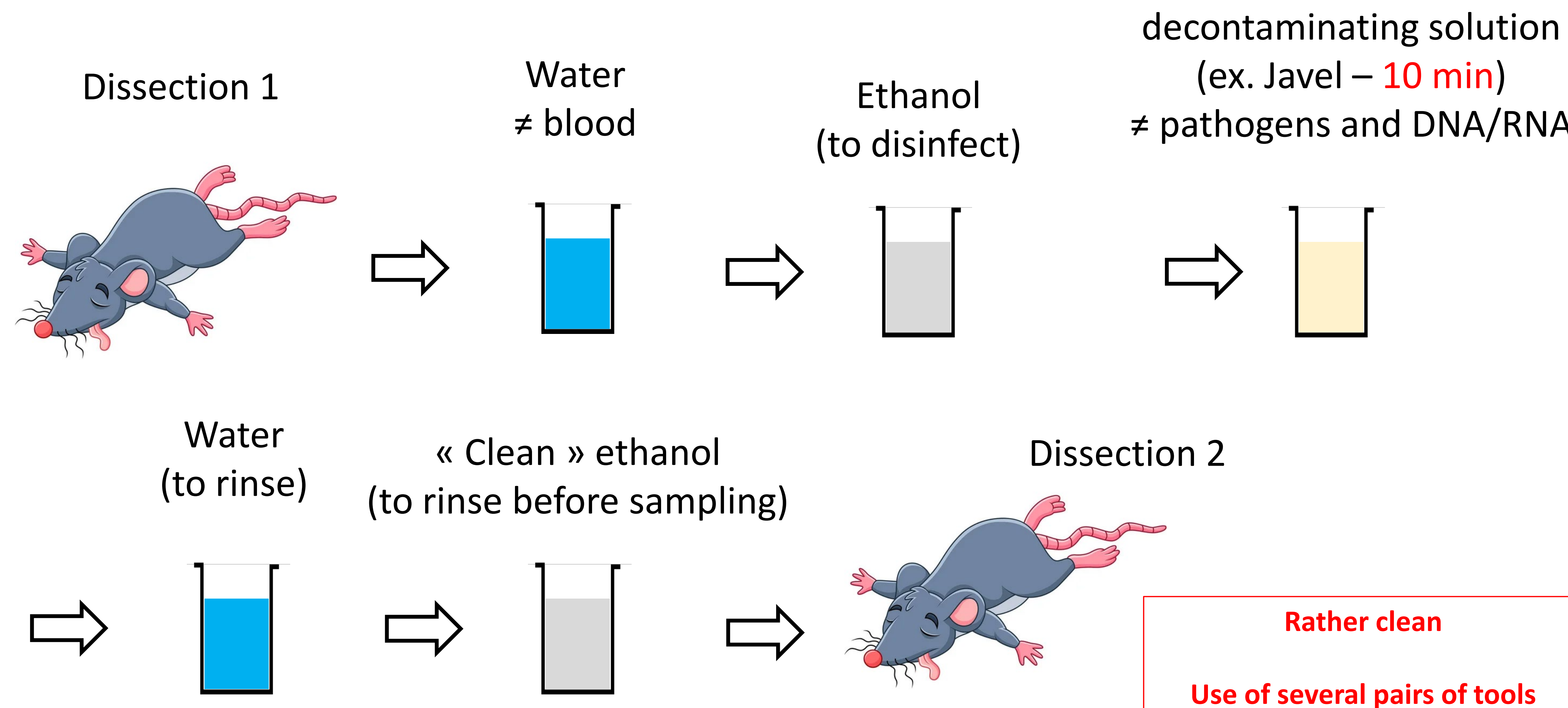
« Clean » ethanol
(to dry)

Flame
(to dry)

Dissection 2

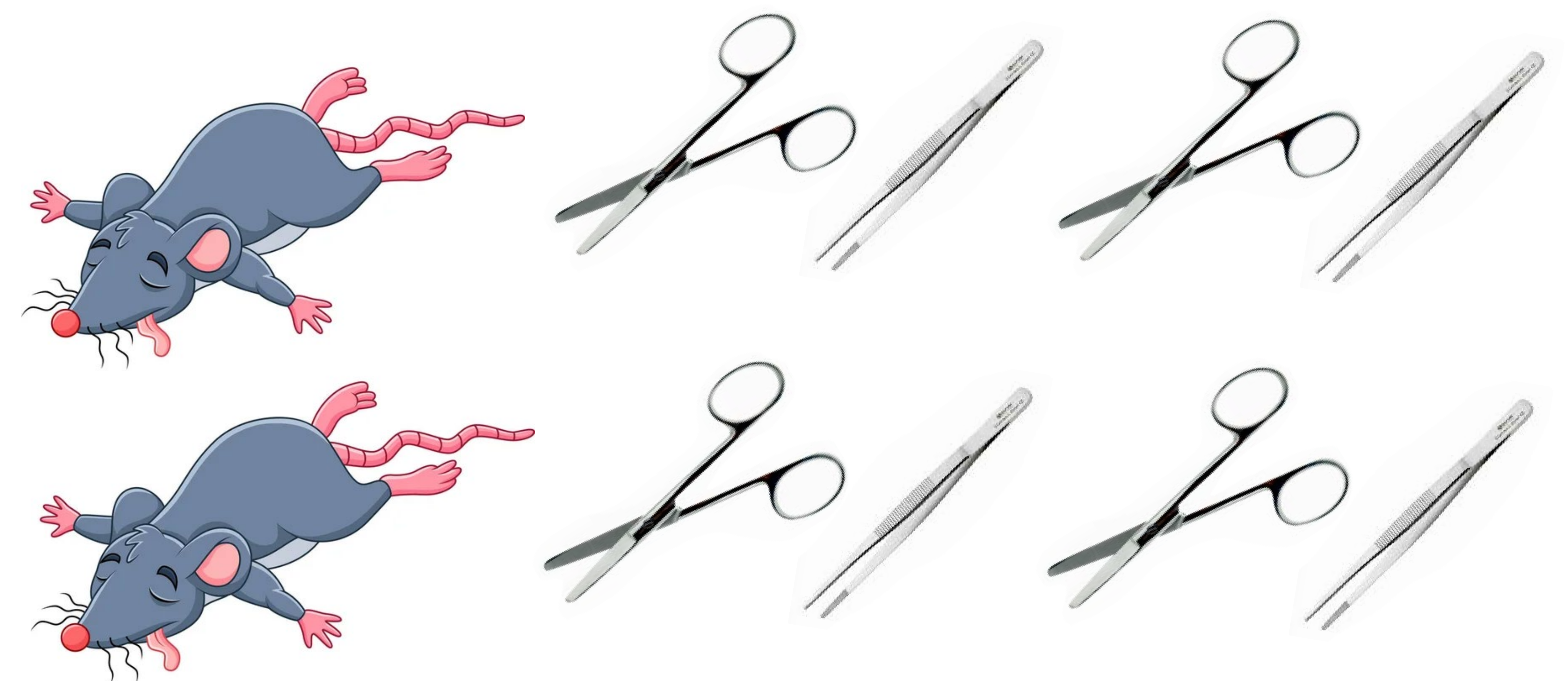
Very clean

Valuable for rapid series (no need to wait in a decontaminating solution)



Dissection 1
Water
≠ blood
Ethanol
(to disinfect)
Desinfecting and
decontaminating solution
(ex. Javel – 10 min)
≠ pathogens and DNA/RNA
Water
(to rinse)
« Clean » ethanol
(to rinse before sampling)
Dissection 2
Rather clean
Use of several pairs of tools
in order to wait long enough
in the decontaminating solution

If a decontaminating solution is used, several pairs of tools are needed for them to stay in it sufficiently long!



- ✓ Dry / non-ethanol instruments into specific solutions
(e.g., RNA-preserving buffers)

- ✓ When sampling digestive tracks, ears, etc … better do it at the end
(i.e., after other inner viscers)

- ✓ NGS experiments are very sensible: in case of organ-specific experiments,
use cleaned organ-specific instruments

2- What to sample:

which organs for which purpose?

Study of mammal reservoirs

- ✓ Taxonomy (e.g., morphology, molecular systematics, voucher specimens)
- ✓ Phylogeography
- ✓ Population genetics
- ✓ Immunology
- ✓ Gene-specific investigation (e.g., resistance to rodenticides, immunogenetics)
- ✓ Functional genomics (i.e., RNA-based transcriptomics)
- ✓ Diet

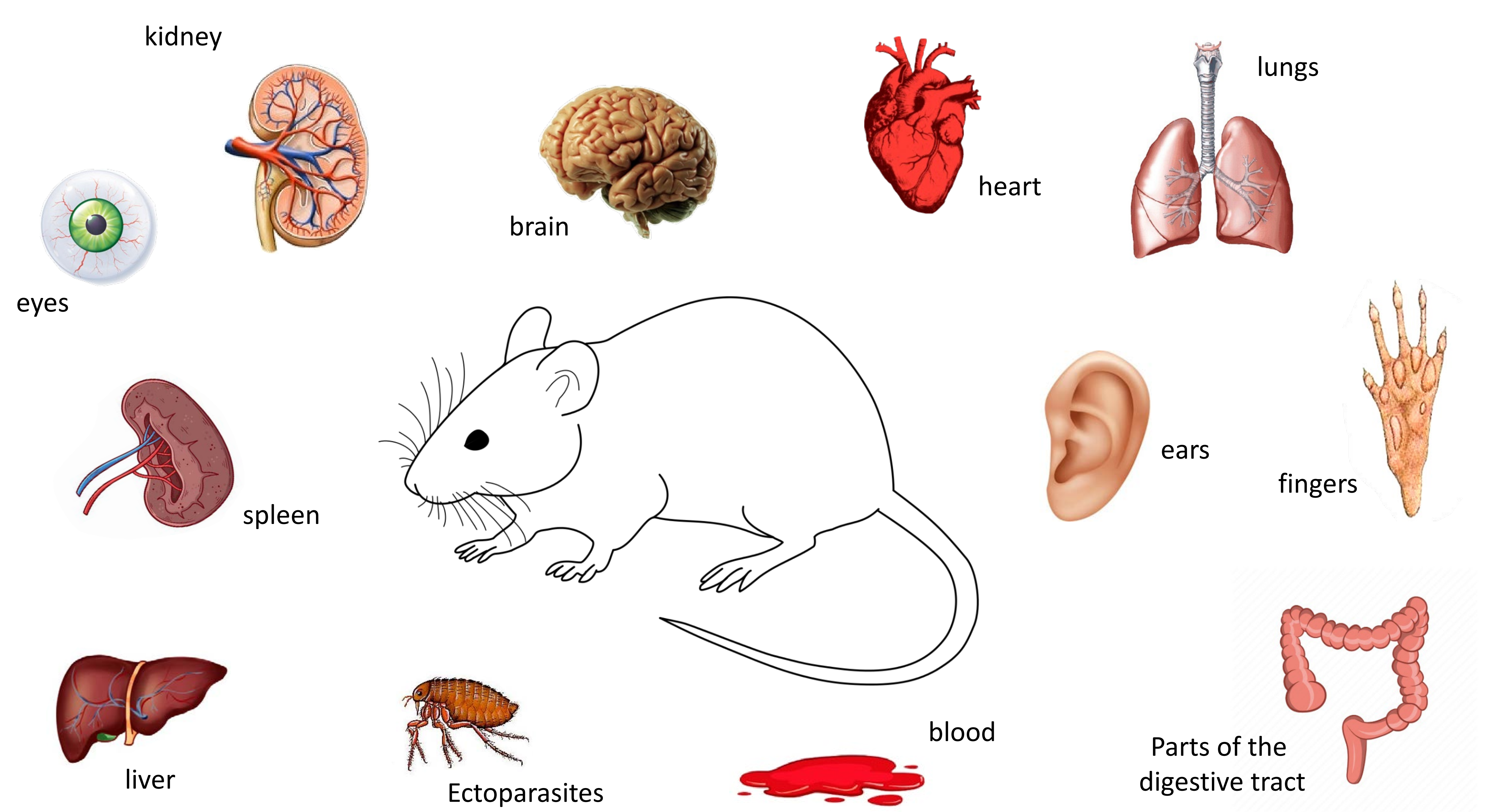
- ✓ Microbiote analyses

Screening / characterization of mammal-borne pathogens

- ✓ Presence/absence (or signatures of previous presence)
- ✓ Direct genotyping (i.e., identification of strain *s. l*.)
- ✓ Population genetics (e.g., *Trypanosoma lewisi*)
- ✓ Gene-specific investigation (e.g., resistance to antibiotics)
- ✓ Microbial culture and phenotyping (e.g., antibioresistance, MAT)

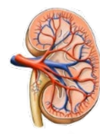
Study of (ecto)parasites

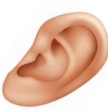
- ✓ Taxonomy
- ✓ Phylogeography
- ✓ Population genetics
- ✓ Gene-specific investigation (e.g., resistance to insecticides)
- ✓ Breeding (e.g., fleas)

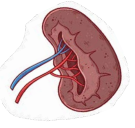

kidney
eyes
brain
heart
lungs
spleen
ears
fingers
liver
Ectoparasites
blood
Parts of the
digestive tract

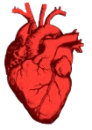
Ethanol 96°

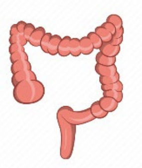
DNA-based study of:

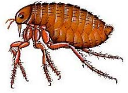
Leptospira 

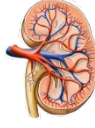
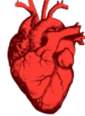
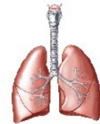
* *Rickettsia* 

Yersinia
Trypanosoma, Leishmania
Pathobiome (meta16S) 

Toxoplasma 

Gastro-intestinal helminths
Gastro-enteritic bacteria (ABMR)
Gut microbiote 

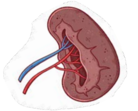
Ectoparasites genetics 

Rodents genetics   

RNA Later
RNA Shield

RNA-based study of:

Hantavirus

Lassa 

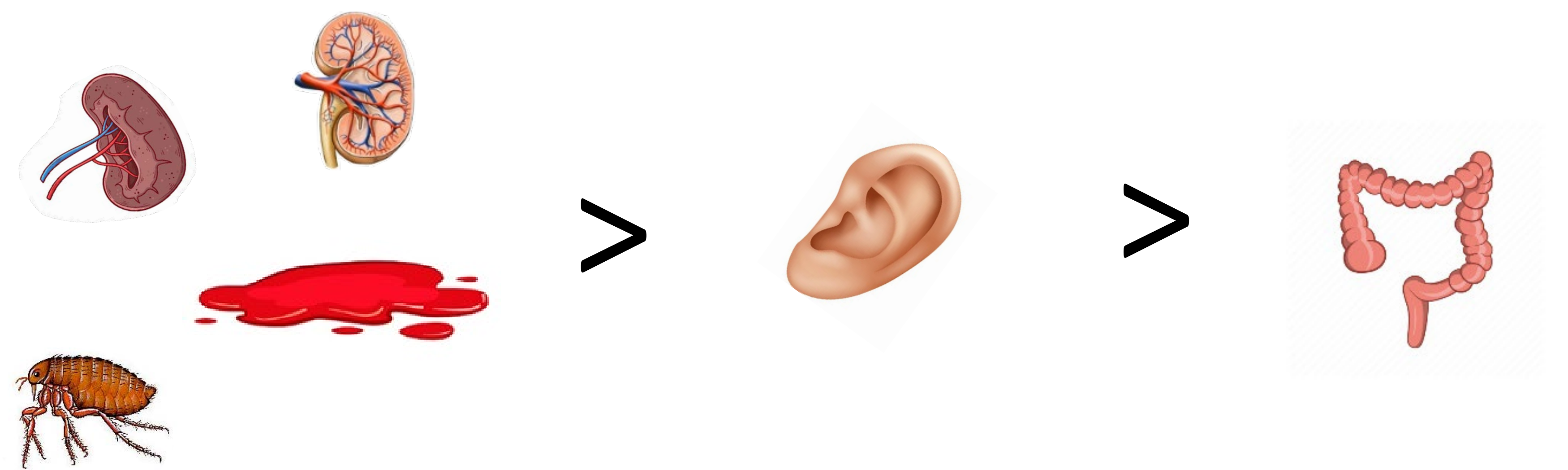
Functional genetics

Dry Blood Spot
Heparin

Serological studies

Specific media
Liquid nitrogen

Living organisms culture



+ screening / analyses spécifiques

Rein car lepto … et génét rongeurs si on fait méta16S sur la rate !!!!

Simple but critical reminders on sampling procedures for future processing in the lab

- ✓ A bit of sample… with a lot of buffer (say, 1:4)
- ✓ Clean the organ, avoid « extra » material (e.g., conjonctive tissues, fat)
- ✓ Incise the organ (without smashing it!) for a better impregnation
- ✓ Make sure the sample is fully submerged in the liquid
- ✓ When lots of blood, it may be useful to change the buffer after 24 hours to avoir excessive dilution

3- Transport and (long-term) preservation:

a few (frequent) examples of constraints

Liquid nitrogen

Availability
Cost
Transport
Risk

RNA preserving buffers

RT then freezing
(Cost)
Viral inactivation?

Ethanol

Flammable
Transport
Quality? (≠pharma)

Formol

Highly toxic
Transport
No MB possible

Ethanol-preserved samples

Use good tubes (i.e., joint-sealed tubes)
Keep ethanol-preserved samples at 4°C
(but check ethanol levels anyway!)

Emma ?

DBS

5 days RT or heat

Long distance transportation of biological samples is getting more and more complicated (and expensive)!

Anticipate (time, money, procedures)

Ethics
Research authorization
Nagoya
Import/export