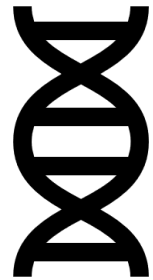


# 病理獸醫師 在基礎研究倫理與實驗室工作 品質扮演的角色與經驗分享



陳彥伯DVM, MSc

2022/10/14@NCHU: CSVP 390



# 目錄

- 題目解析
- 自我介紹
- 基礎研究與實驗室的各項病理工作及研究倫理注意事項
- 獸醫師在基礎研究的動物倫理及品質所扮演的角色
- 病理獸醫師在基礎研究與實驗室工作介紹
- 病理獸醫師經驗分享

# 題目分析

- 病理獸醫師在[基礎研究與實驗室工作]扮演的角色與經驗分享
- 病理獸醫師在基礎研究倫理與實驗室工作品質扮演的角色與經驗分享

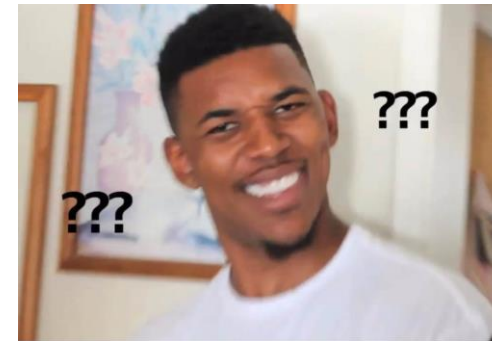
- 基礎研究倫理與實驗室工作品質

- 病理獸醫師?

- IRB? (Institutional Review Board)

- Research integrity?

- GLP? Good Laboratory Practice? ISO17025/ISO9001?





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# 陳彥伯簡介

- 現職：台灣小鼠診所 病理獸醫師 2021-
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  - 病理獸醫師 碩聯動物醫院 2019/7-2020/10
  - 研究助理 中央研究院 基因體中心 幹細胞實驗室2015/7-2017/3
  - 研究助理 中興大學獸醫系電子顯微鏡實驗室2013/9-2015/4
- 專業證照：病理獸醫師 2016/11
- 專長：單車橫跨歐亞(B2P project), 2017-2018



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# 基礎研究與實驗室的工作及研究倫理注意事項

- 人體試驗倫理委員會(Institutional Review Board,簡稱IRB)
- 同儕、編輯審查 Editorial review and peer review/ revision
- 學術倫理 Research integrity

# 研究倫理與人體試驗倫理委員會

- 研究倫理(research ethics)廣義地說，乃是探討一切研究行為所涉及的倫理議題，因此舉凡研究設計、執行、學術或研究成果發表所涉及的倫理議題，均為其探討對象。
- 「人體試驗倫理委員會(Institutional Review Board，簡稱IRB)」是為確保人體試驗或研究符合科學與倫理適當性，所設立的審查單位。
- 紐倫堡守則
- 「赫爾辛基宣言」





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# 獸醫師在基礎研究的動物倫理及品質所扮演的角色

- 獸醫相關：實驗室/實驗動物/實驗動物操作實驗室
  - IACUC, Institutional Animal Care and Use Committee, 實驗動物照護及使用委員會
  - AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care International 國際實驗動物管理評鑑及認證協會
  - GLP, Good Laboratory Practice 藥物非臨床研究品質管理規範
- 病理獸醫師：
  - 生技及醫藥產業、CRO
  - 大型研究單位
  - 動物醫院
  - 政府防疫單位
  - 大學及教育單位



# Institutional Animal Care and Use Committee

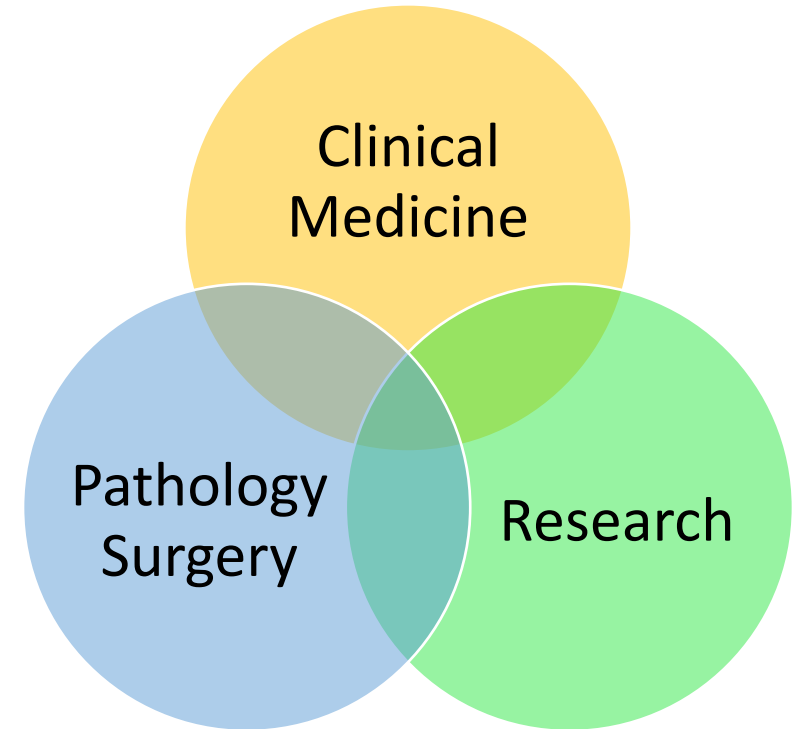
## 實驗動物照護及使用委員會

- 研究涉及使用動物時的規範組織
- 自行監督程序、適當的飼養管理、獸醫照顧計畫、健全的動物房舍與記錄保存等
- 動物實驗管理小組：必須至少有5名委員，其中要有一名獸醫師、一名與機構無密切關係的人員、和一名非生物專業的人員。
- 管理小組至少每6個月要檢查所有動物場所與研究機構一次
- IACUC必須向機構首長(農委會)提出一份書面的檢查報告
- 如試驗中會造成動物痛苦時，必須諮詢獸醫師的意見，以便實施麻醉、止痛或鎮靜。研究結束時，動物如須被犧牲，要採行符合人道的安樂死。

1-14	排水	有良好的排水設備，必要時應穿著防滑鞋。	3.2.1(6)		
1-15		長期不使用之排水系統，得加蓋和密封，以避免濁氣、害蟲或其他污染物之逆流與侵入。	3.2.1(6)		
1-16	遮蔽	飼養於戶外的圍籬設施時，提供可避免動物暴露在極端氣溫或其他惡劣天氣變化的欄舍與附屬構造或者照養機制。	<b>3.6.1(1)</b>		
1-17		遮蔽物之設置有足夠的空間以容納所有的動物。	3.6.1(2)		
1-18		有足夠的通風避免廢棄物及過量濕氣堆積。	3.6.1(2)		
1-19	照明及	提供足夠的照度以滿足動物生理、福祉及現場操作管理需求。	3.3.1(3)		
1-20	電力供應	動物房內得採用定時控制之照明系統，提供正常的光照週期，並定期檢視系統性能。	3.2.2(3)		
1-21		有備份電力供應系統或緊急發電系統供設施或支援性功能得以持續運作。	3.2.2(2)		
1-22	溫溼度	動物被圈養在適合的溫溼度範圍內。	3.3.1(1)		
1-23	空間	飼育空間應足以讓動物表現正常姿態、隨意調整姿勢、攝食與飲水、不會碰觸到圍籬、籠壁或籠頂，提供動物可遠離糞尿沾污的休息區域。	<b>3.4.1(1)</b>		
1-24		符合常見實驗動物的最小建議空間需求	<b>3.4.1(1)</b>		
1-25	噪音及震動	考量噪音控制的措施，並嘗試去減少震動的產生。	3.3.1(4)		
1-26	動物識別	動物識別卡上應記錄動物來源、品種或品系、相關日期資料(如接收日期、出生日期等)、負責研究人員姓名與聯絡資料及動物實驗申請表編號等資訊。	<b>3.14.1(1)</b>		
1-27	功能性設施	動物設施得規劃一個特定的公用區域作為清洗消毒飼育籠具及附屬配件之用。	4.1		
1-28		規劃無菌手術功能區域，手術設施與其他區域得有充足的空間區隔，以減少不必要的動線交錯狀況，降低污染風險。	4.2		
1-29		動物造影區域應規劃麻醉劑與攜帶氣體供應系統、麻醉廢棄的清除，以及全程動物監控之機制。	<b>4.3.1(1)</b>		
1-30		設置核磁共振造影設備區域，應裝置氧氣偵測器及增加房間通風，以排除填充冷劑或冷劑蒸發時產生的惰性氣體，導致人員和動物窒息的機率。	<b>4.3.1(2)</b>		
1-31		規劃行為研究的設施時，應注意在設施設計、建構、設備及運作等各方面是否會對測試動物產生不適當的感官刺激。	<b>4.4.1(1)</b>		

# Laboratory Animal Medicine Vet and Job Description

- LAM -Provide for the health and well-being of animals
- Comparative Medicine Research -Study of biology and disease in animal models to improve human and animal health
- Science –study directors or project leaders in fields of applied medicine
- 工作內容如下：
  1. Quality management of animal facility
  2. Quarantine, veterinary care and disease surveillance
  3. Evaluation, execution or supervision for LA surgery, anesthesia, analgesia and euthanasia
  4. Routine administrative work
  5. EVERYTHING assigned by supervisor



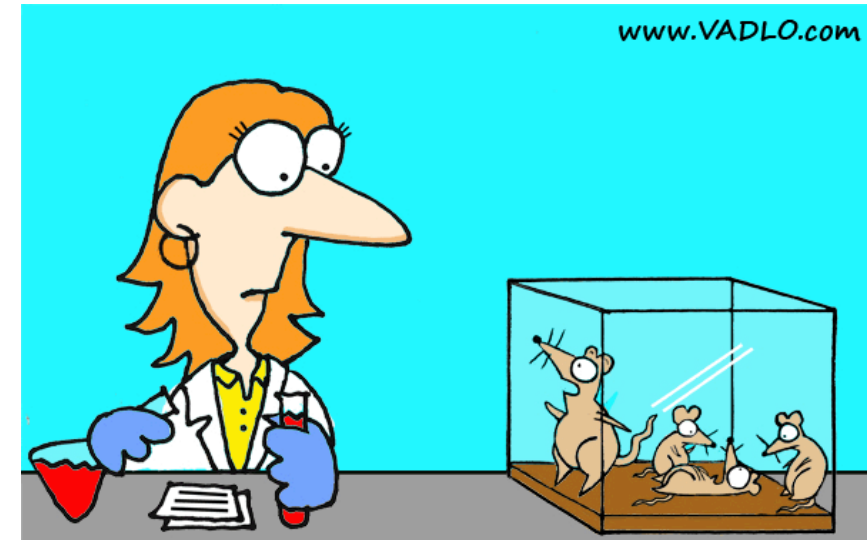


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- **病理獸醫師在基礎研究與實驗室工作介紹**
- 病理獸醫師經驗分享

# 病理獸醫師在基礎研究與實驗室工作介紹

- Need a Pathologist? Or just need someone to do pathology things?
  - A vet with pathologic knowledge
  - Technique: histologist
  - Who can tell me what's it in microscope?
- Do (comparative) pathology research
  - Human catch viruses, who can catch mice for me?
  - Animal experiment & analysis (animal models)
  - Various fields: cardiovascular, cancer, metabolism
  - Wet lab techniques: IHC, genotyping etc.
  - Oh, well, so you know how to read slides?



“Hey, my pup is sick. Are you a real doctor or just PhD?”

# 基礎研究與實驗室工作分享

- 中央研究院 基因體中心 幹細胞實驗室 研究助理2015-2017
- Establishment animal model of circulating tumor cell (pancreatic adenocarcinoma and small cell lung cancer); 建立循環腫瘤幹細胞
- Evaluation of diabetic drug on pancreas regeneration *in vivo*; 糖尿病藥物對胰臟再生
- Evaluation iPSC-based cell therapeutics for diabetic complications; 以iPSCs 治療糖尿病症候群
- Evaluation of mustard anti-colon cancer drugs toxicity 芥子素類藥物抗大腸癌之毒性
- Discovered relationship between diabetic status and pancreatic cancer by transgenic mice model; 胰臟癌(PDCA)與T2DM基轉小鼠培育



# 基礎研究與實驗室工作分享

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# Literature Review



doi:10.1111/j.1440-1746.2011.06938.x

GASTROENTEROLOGY

## Diabetes mellitus correlates with increased risk of pancreatic cancer: A population-based cohort study in Taiwan

Kuan-Fu Liao,<sup>\*,\*\*</sup> Shih-Wei Lai,<sup>†,¶</sup> Chia-Ing Li<sup>†,\*,\*\*</sup> and Wen-Chi C

\*Department of Internal Medicine, Taichung Tzu Chi General Hospital, and <sup>†</sup>School of Medicine Chinese Medicine, China Medical University, and Departments of <sup>¶</sup>Family Medicine and <sup>\*\*</sup>N University Hospital, Taichung, and <sup>\*\*</sup>School of Medicine, Tzu Chi University, Hualien, Taiwan



**NIH Public Access**  
**Author Manuscript**  
*Mol Carcinog.* Author manuscript; available in PMC 2013 January 1.

Published in final edited form as:  
*Mol Carcinog.* 2012 January ; 51(1): 64–74. doi:10.1002/mc.20771.

### Diabetes and Pancreatic Cancer

**Donghui Li\***  
Department of Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

### Abstract

Type 2 diabetes mellitus is likely the third modifiable risk factor for pancreatic cancer after cigarette smoking and obesity. Epidemiological investigations have found that **long-term type 2 diabetes mellitus is associated with a 1.5- to 2.0-fold increase in the risk of pancreatic cancer.** A

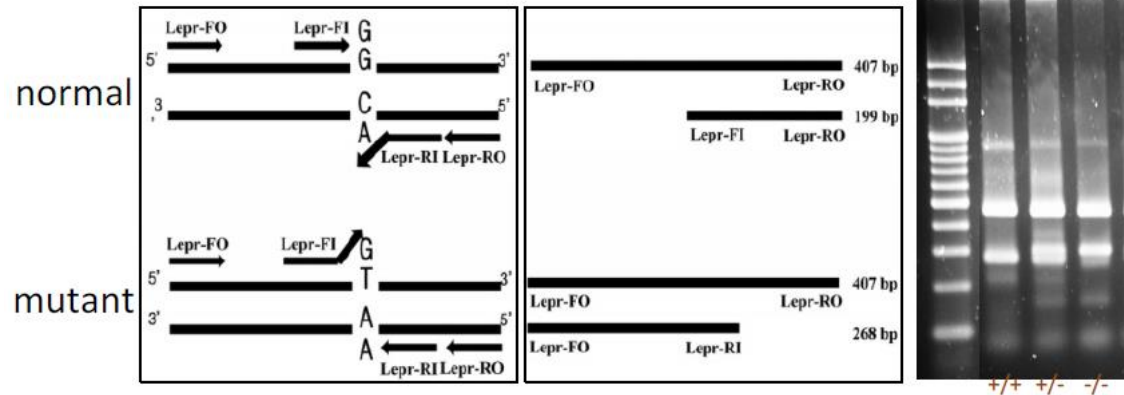
# Relationship Between T2DM and Pancreatic Cancer

- Diabetes may increase the risk of pancreatic cancer at 40%.
- The purpose of our experiment is to find out how pancreatic tumor develops under type II diabetes mellitus (T2DM) .
- Pancreatic ductal adenocarcinoma (PDAC)
- db/db mouse: BKS.Cg-*Dock7*<sup>m+/+</sup>*Lepr*<sup>dbJ</sup>
- Genotyping and phenotyping: BW, BG, glucose tolerance test
- B6 strain derived pancreatic cancer with luciferase-GFP, IVIS system
- Patient derived orthotopic xenograft PDOX: intra-pancreatic injection

# Genotyping and Experimental Design

- db/db mice contains G to T point mutation
- B6 mouse with oncogene mutation induced pancreatitis with Cerulein

## ① Genotyping (ARMS-PCR)



- Pancreatic neoplastic cell with Luciferase-GFP
- Engraftment with  $10^3$  cell in pancreas of db/db, wild type and B6 mice

# Physiological Changes

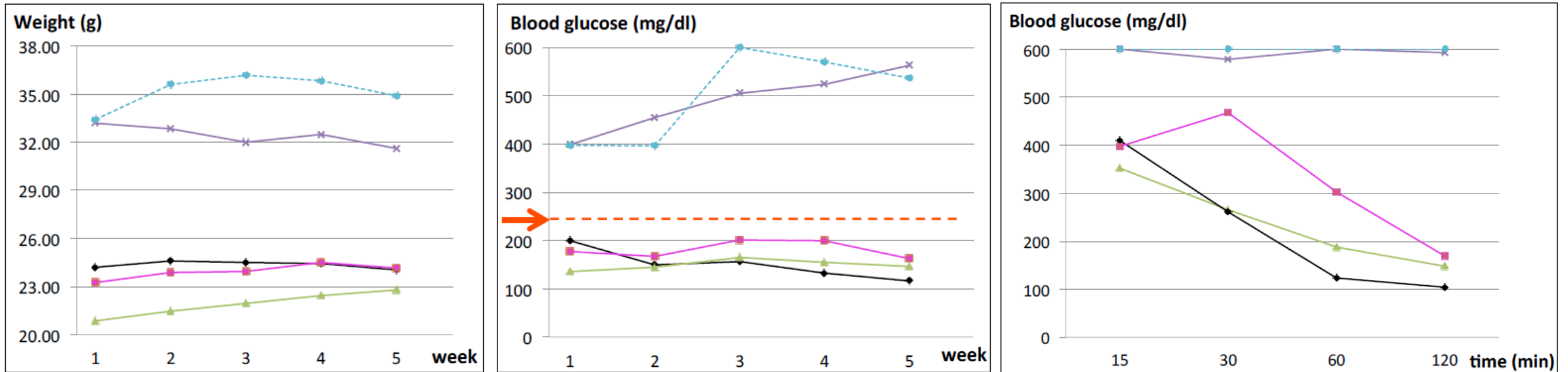
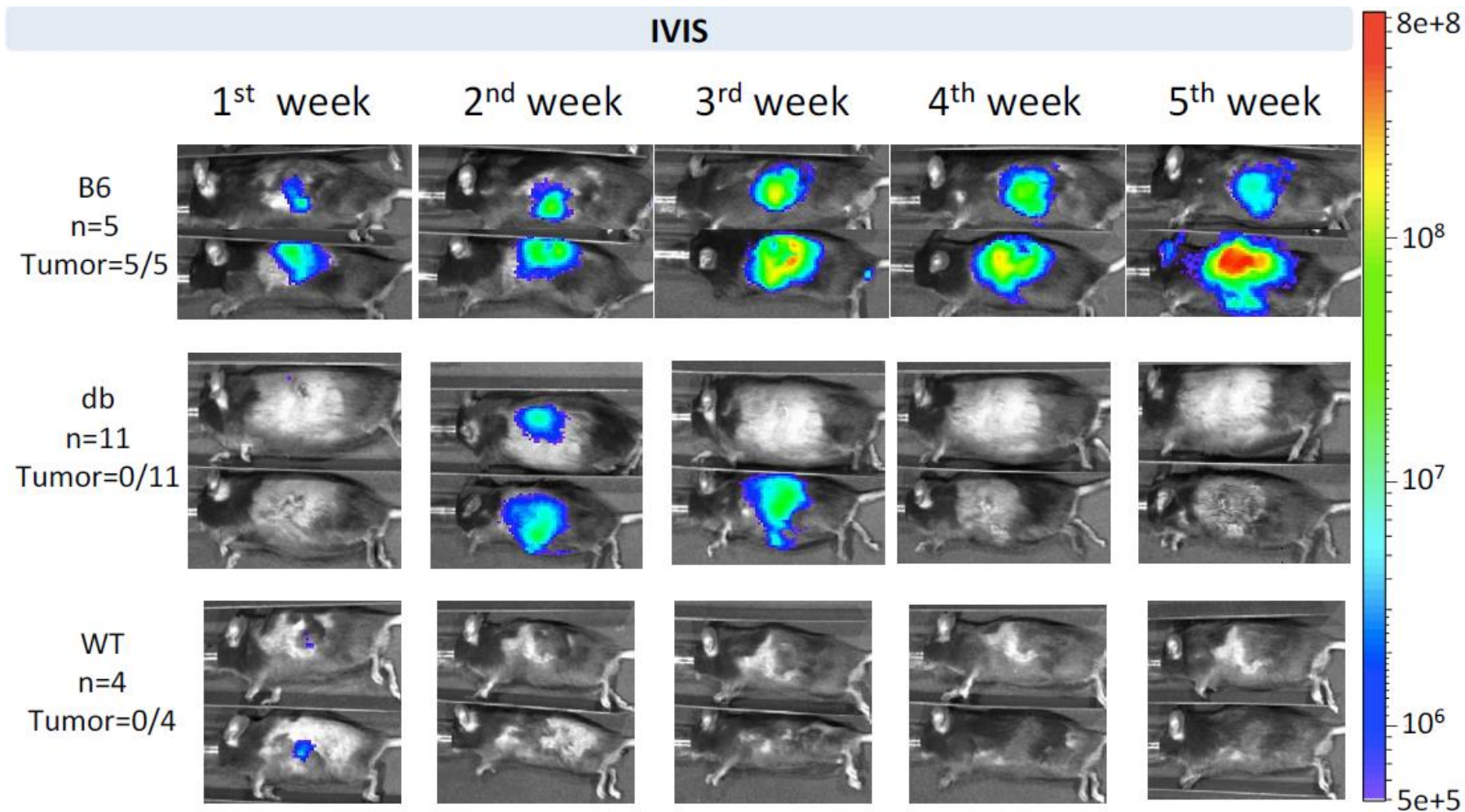


Fig 1. -●- db (ctrl) -x- db (pan18) -■- B6 (ctrl) -▲- WT (pan18) -◆- B6 (pan18)

a) Body weight per week. b) Blood glucose per week, → 250mg/dl is the cut off value. c) Glucose tolerance test is carried after 6hrs fasting and by giving 1mg/g glucose intraperitoneally.

# IVIS 活體冷光螢光影像分析系統



# Complete Blood Count

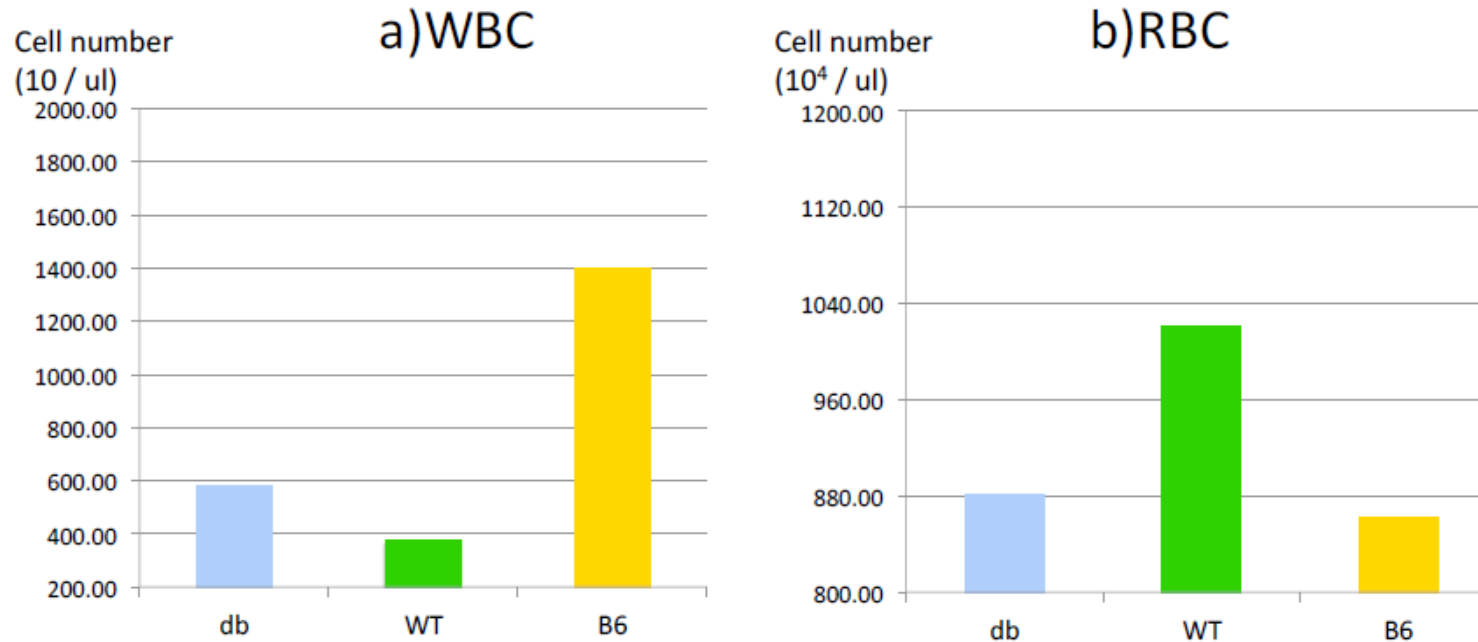


Fig 3. Comparisons of number of blood cells between db/db, wild type and B6 mice. a) db: neutrophil ↑, Monocyte ↑ ; WT: monocyte ↑, neutrophil ↑ ; B6: Monocyte ↑ b) B6: HGB ↓

# Pathology Examination

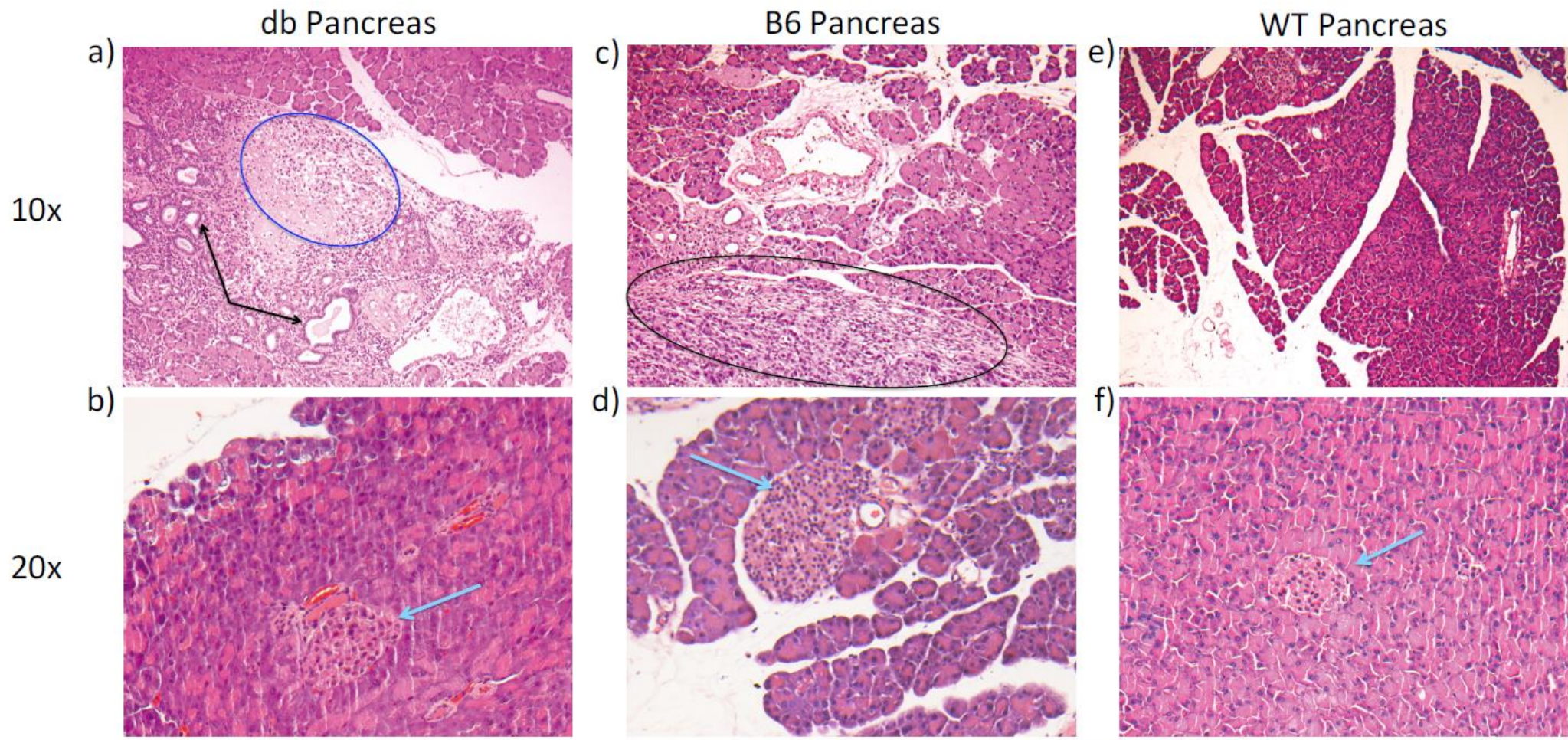


Fig 2. H&E histology slides of pancreas. a)  $\longrightarrow$  = tumor cells,  $\bigcirc$  = inflammation ; c) tumor with pancreas ; b), d), f) islet of langerhans



# The “I TOLD YOU” MOMENT

## Major Histocompatibility Complex (MHC): Mouse

[View previous versions](#)

Patrick M Stuart

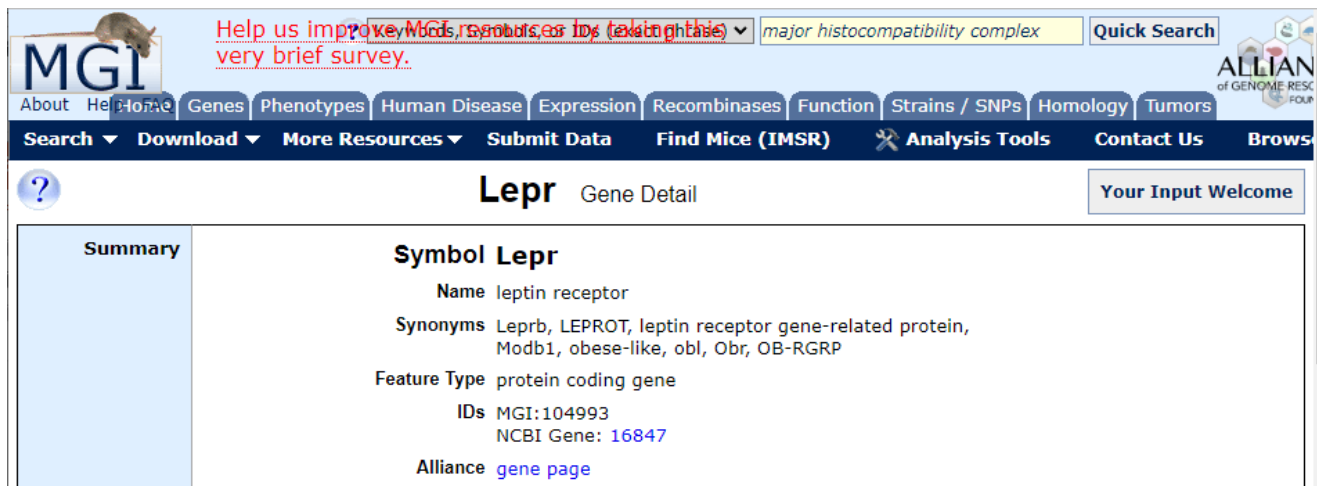
First published: 15 June 2015 |

<https://doi.org/10.1002/9780470015902.a0000921.pub4> | Citations: 3

Based in part on the previous version of this eLS article ‘Major Histocompatibility Complex (MHC): Mouse’ by Roger W Melvold.

Table 1. H2 haplotypes of some common laboratory mouse strains

Strain	H2 haplotype	Functional H2 loci: classes I and II						
		H2-K	H2-Ab	H2-Aa	H2-Eb	H2-Ea	H2-D	H2-L
<i>Standard</i>								
C57BL/6, C57BL/10	<i>H2<sup>b</sup></i>	<i>K<sup>b</sup></i>	<i>Ab<sup>b</sup></i>	<i>Aa<sup>b</sup></i>	<i>Eb<sup>b</sup></i>	<i>-<sup>a</sup></i>	<i>D<sup>b</sup></i>	<i>-</i>
BALB/c, DBA/2	<i>H2<sup>d</sup></i>	<i>K<sup>d</sup></i>	<i>Ab<sup>d</sup></i>	<i>Aa<sup>d</sup></i>	<i>Eb<sup>d</sup></i>	<i>Ea<sup>d</sup></i>	<i>D<sup>d</sup></i>	<i>L<sup>d</sup></i>
C3H, CBA, AKR	<i>H2<sup>k</sup></i>	<i>K<sup>k</sup></i>	<i>Ab<sup>k</sup></i>	<i>Aa<sup>k</sup></i>	<i>Eb<sup>k</sup></i>	<i>Ea<sup>k</sup></i>	<i>D<sup>k</sup></i>	<i>-</i>
B10.M	<i>H2<sup>f</sup></i>	<i>K<sup>f</sup></i>	<i>Ab<sup>f</sup></i>	<i>Aa<sup>f</sup></i>	<i>-</i>	<i>-</i>	<i>D<sup>f</sup></i>	<i>-</i>
DBA/1, SWR	<i>H2<sup>g</sup></i>	<i>K<sup>g</sup></i>	<i>Ab<sup>g</sup></i>	<i>Aa<sup>g</sup></i>	<i>-</i>	<i>-</i>	<i>D<sup>g</sup></i>	<i>L<sup>g</sup></i>
SJL	<i>H2<sup>s</sup></i>	<i>K<sup>s</sup></i>	<i>Ab<sup>s</sup></i>	<i>Aa<sup>s</sup></i>	<i>Eb<sup>s</sup></i>	<i>Ea<sup>s</sup></i>	<i>D<sup>s</sup></i>	<i>-</i>



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major histocompatibility complex Quick Search

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**Lepr** Gene Detail Your Input Welcome

**Summary**

**Symbol Lepr**

**Name** leptin receptor

**Synonyms** Leprb, LEPROT, leptin receptor gene-related protein, Modb1, obese-like, obl, Obr, OB-RGRP

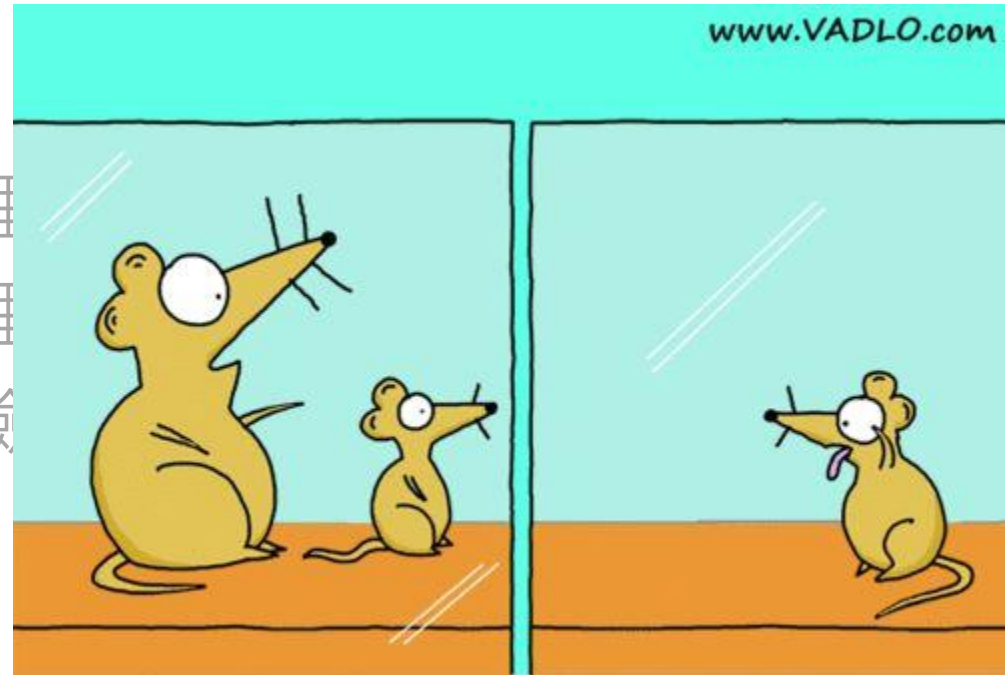
**Feature Type** protein coding gene

**IDs** MGI:104993  
NCBI Gene: 16847

**Alliance** [gene page](#)

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“Don’t play with him, he is **Wild Type**.”

# Three Quality Indicators in Quality of the Report:

- **Thoroughness:** identification of every lesion present in a particular organ or tissue, including spontaneous background lesions
- **Accuracy:** ability to make, and precisely communicate, correct diagnoses. (Nomenclature of lesion)
- **Consistency** uniform use of a specific term to record a defined lesion and implies that the same diagnostic criteria are being followed for each type of diagnosis. (diagnostic drift; semiquantitative or quantitative severity)

Cynthia Shackelford. 2002. Qualitative and Quantitative Analysis of Nonneoplastic Lesions in Toxicology Studies. Toxicol Pathol 2002, 30: 93-96

# Some commonly used severity grading schemes

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## Grading scheme I

- 0 = Not present
- 1 = Minimal (<1%)
- 2 = Slight (1–25%)
- 3 = Moderate (26–50%)
- 4 = Moderately Severe/high (51–75%)
- 5 = Severe/high (76–100%)

## Grading scheme II

	A	B
Grade 1 = Minimal	(<10%)	(0–25%)
Grade 2 = Mild	(10–39%)	(26–50%)
Grade 3 = Moderate	(40–79%)	(51–75%)
Grade 4 = Marked	(80–100%)	(76–100%)

## Grading scheme III

- Grade 1 = Minimal
  - Grade 2 = Slight (same as mild)
  - Grade 3 = Moderate
  - Grade 4 = Marked (same as severe)
  - Grade 5 = Massive (same as very severe)
-



# Current Work as a Veterinary Pathologist in Taiwan Mouse Clinic

- Inner Service:
  - Laboratory Animals (mouse, rat and hamster) disease diagnosis
    - Non-infectious disease (spontaneous, senile)
    - Severely immunodeficient mice and germ-free mice
    - Histopathology evaluation in animal model: arthritis, neoplasia,
  - Standard Operating Protocol (SOP) of animal facility, pathology and toxicopathology
  - GLP preparation
  - Pathogenesis study
- Open service:
  - Laboratory animal necropsy, sampling and subsequent processing
  - Experimental pathology: translation medicine, pharmacology study on various therapies
  - Genetically Engineered Models



- Taiwan Mouse Clinic (TMC)

## TMC 設立目標

- 提供高品質與多元化的小鼠表現型分析服務
- 建立各式樣藥物安全性及藥效評估的標準化及客制化套裝服務，以協助學界與產業界新藥之開發。

# Mouse Hotel

## 設施服務:

SPF 大、小鼠代養服務

SPF 小鼠淨化服務

動物房出租之管理服務

無菌鼠代養服務

腸道菌接種服務

## 設備:

(1) ABSL1 等級獨立SPF動物房，全區域IVC (individually ventilated cage)飼養

(2) BSC (biosafety cabinet)

(3) 自動化清洗與滅菌設備

## Double-tier Isolator

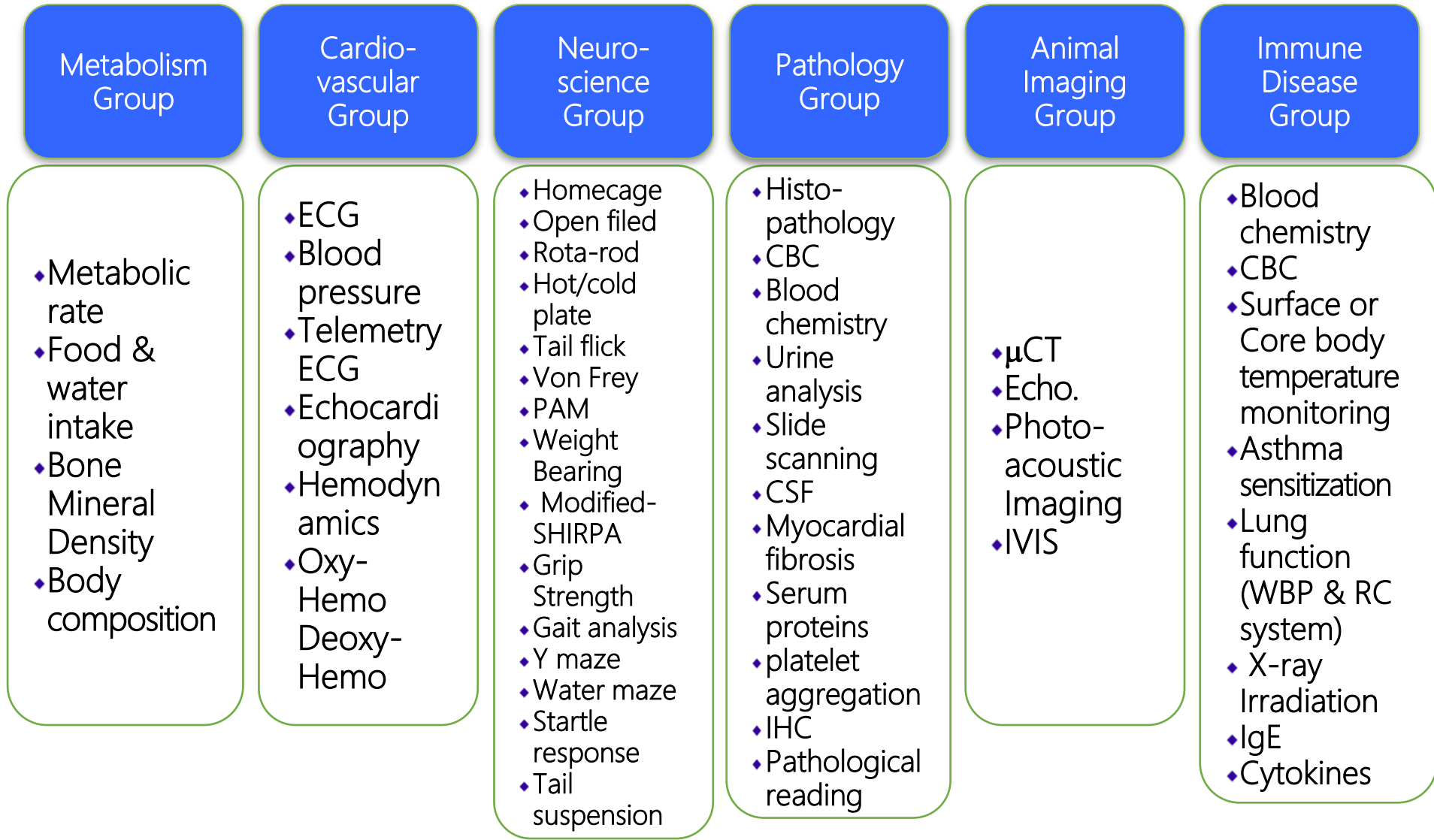


## Anaerobic Chamber



- Microbiome Studies
- Anaerobic microbiology research

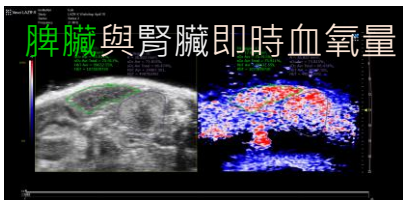
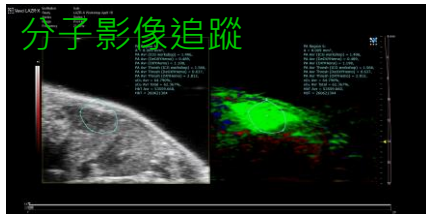
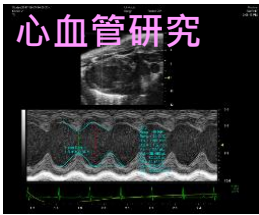
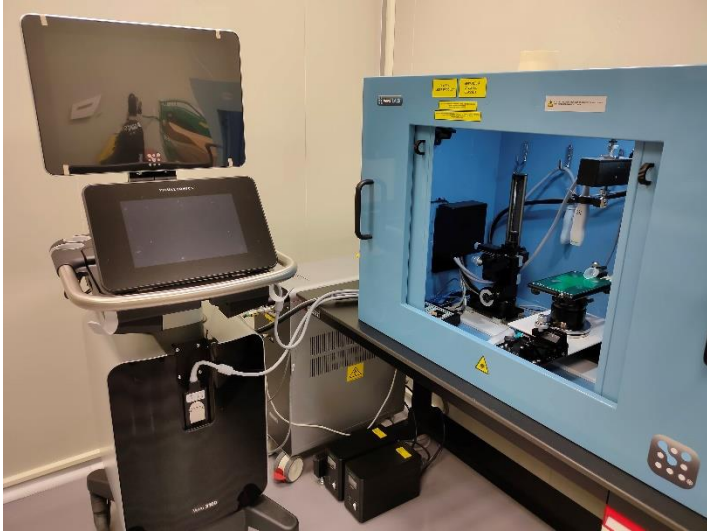
# • Phenotyping services



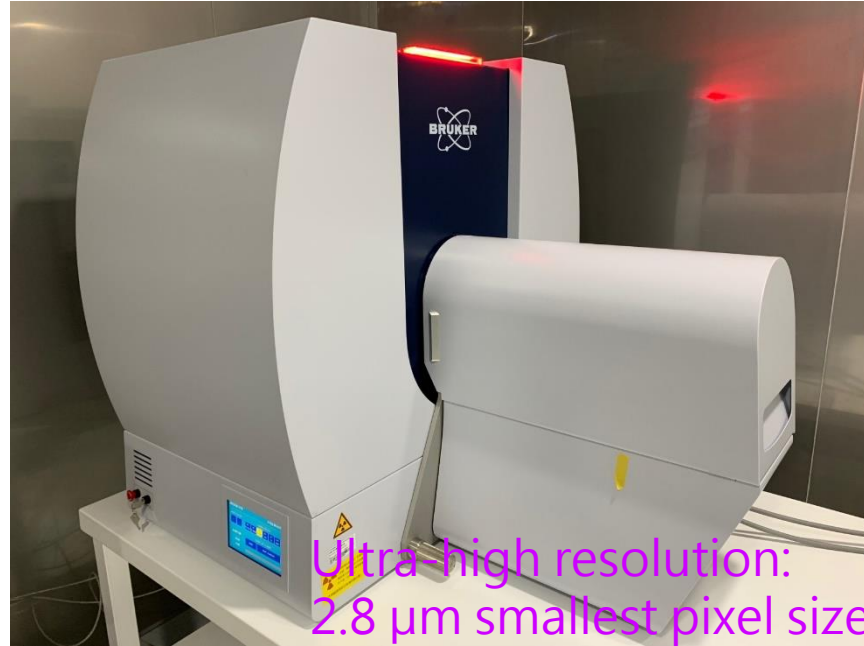
- ### Routes of administration
- i.v.
  - i.m.
  - i.p.
  - s.c.
  - Gavage
  - Intratracheal
  - **Inhalation (Tower)**



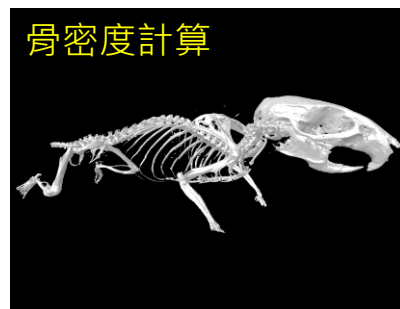
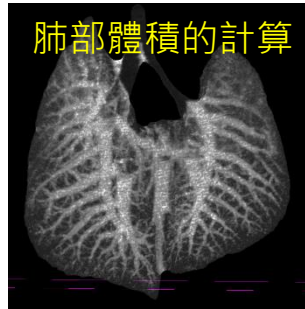
## 全頻雷射光聲超音波系統 (Phoacoustic Imaging System)



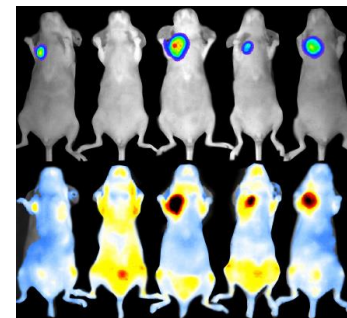
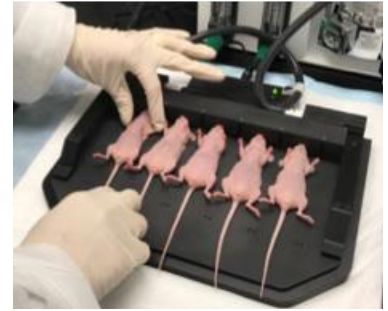
## 電腦斷層掃描 (Micro CT, SkyScan 1276)



Ultra-high resolution:  
2.8  $\mu\text{m}$  smallest pixel size



## 高靈敏螢冷光分子影像系統 IVIS<sup>®</sup> Lumina S5 imaging system



4T1-Red-Fluc orthotopic breast tumor:  
(top) bioluminescent signal  
(bottom) fluorescence detection

- Pharmacodynamic

efficacy

Dose-Response

In vivo studies

- Safety Pharmacology

Core battery:

-CNS

-Respiratory

-Cardiovascular

- Toxicology

Toleration

Acute

Sub-chronic

Chronic

Genetic

*reproductive*



## ■ A xenograft or allograft tumor model

Models: subcutaneous, orthotopic, and systemic models

Cancer types: Breast, Lung, Pancreatic, Colon, Melanoma, Prostate, Liver (Ultrasound-guided intrahepatic injection), Ovarian

## ■ Ovalbumin (OVA)-induced asthma mouse model

## ■ Pain model

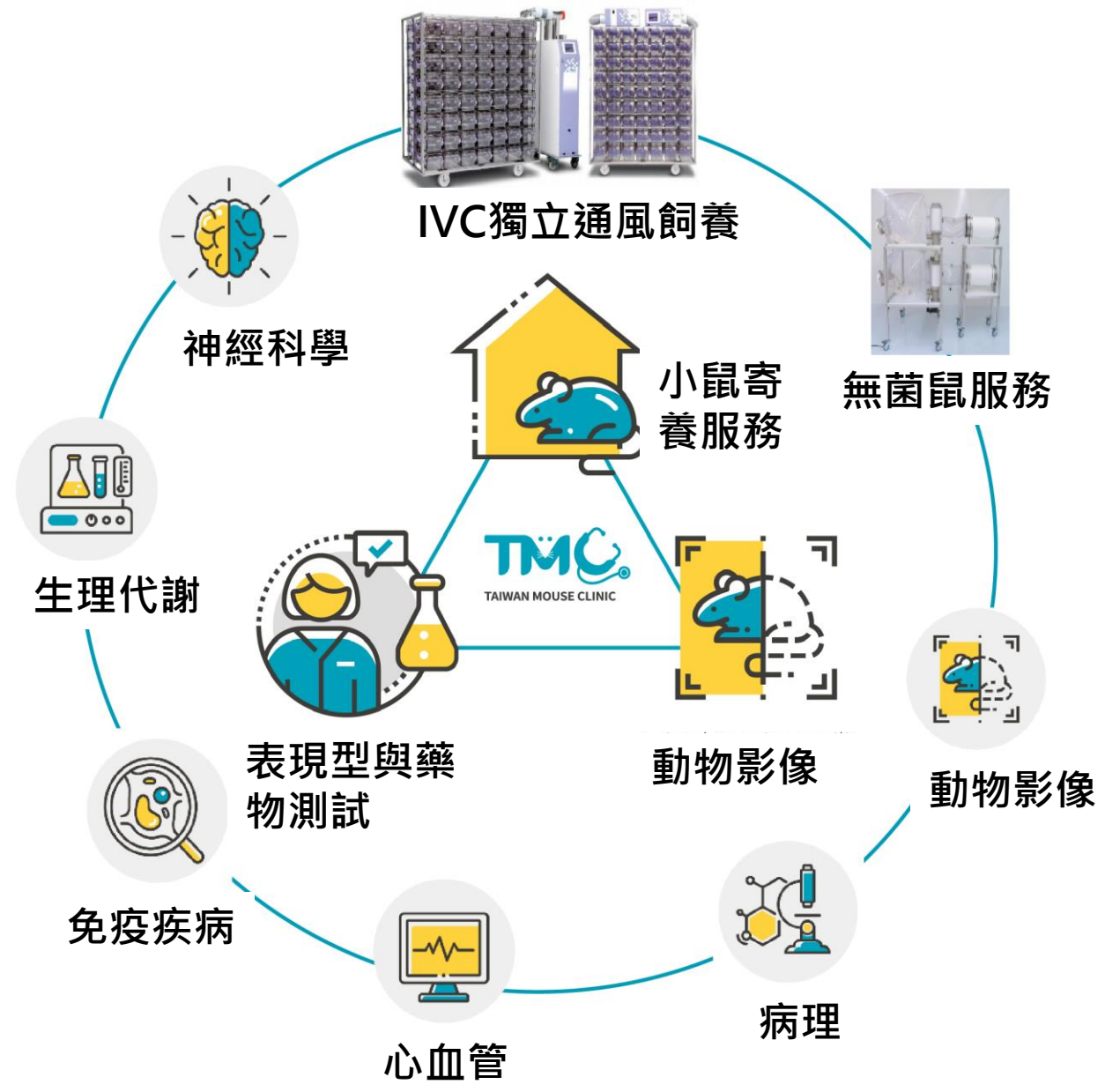
Fibromyalgia (纖維肌痛症), Formalin-induced pain, Acetic acid-induced visceral pain, Neuropathic pain induced by spared nerve injury (坐骨神經分支選擇結紮切斷) or oxaliplatin/ cisplatin

## ■ Addiction model: morphine addiction

## ■ Complete Freund's adjuvant (CFA)-induced rheumatoid arthritis (in both mouse and rat models)

## ■ STZ (streptozotocin) induced diabetes

## ■ Diet-induced (high fat diet, 60 Kcal%) obesity





Thanks for your attention!  
感謝聆聽!



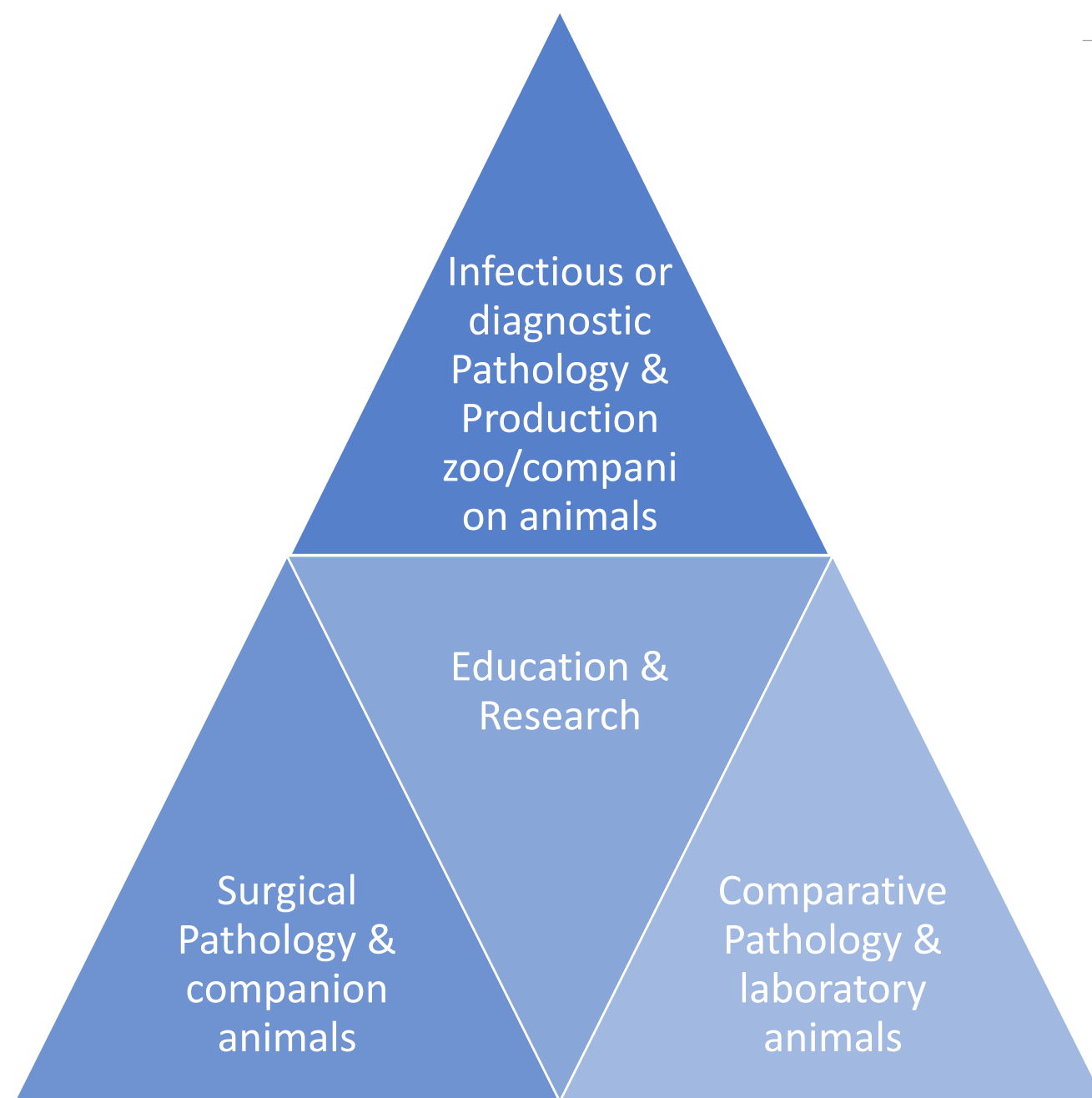


## Q&A時間



# 目錄

- 題目解析
- 自我介紹
- 基礎研究與實驗室的各項病理工作及研究倫理注意事項
- 獸醫師在基礎研究的動物倫理及品質所扮演的角色
- 病理獸醫師在基礎研究與實驗室工作介紹
- 病理獸醫師經驗分享





# What's your Dx? What is it?

