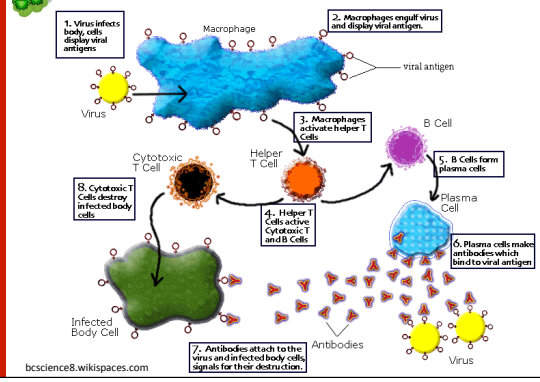
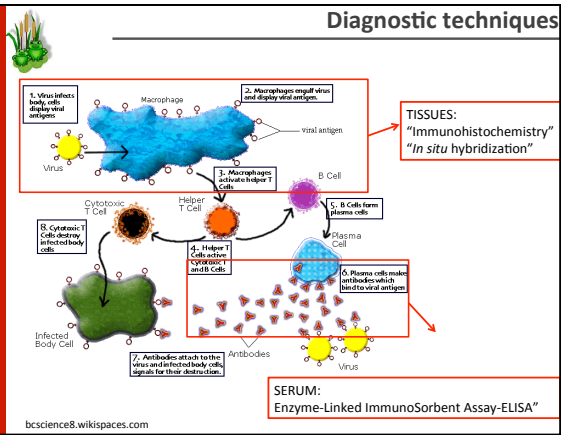
Diagnostic techniques: IHC/*In situ* hybridization/ELISA

Dr. Ana Balseiro
-SERIDA-

Immune response



Diagnostic techniques



TISSUES:
"Immunohistochemistry"
"*In situ* hybridization"

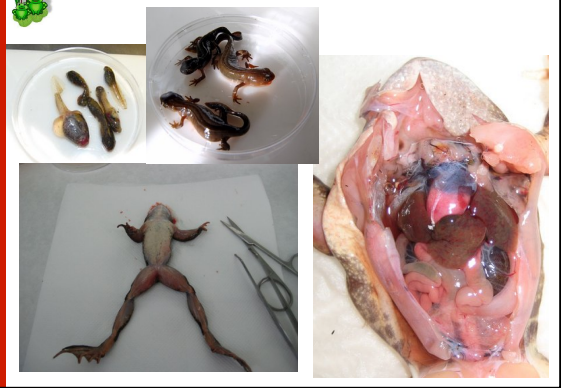
SERUM:
Enzyme-Linked ImmunoSorbent Assay-ELISA"

Immunohistochemistry

OBJECTIVE

Detect antigen in tissues

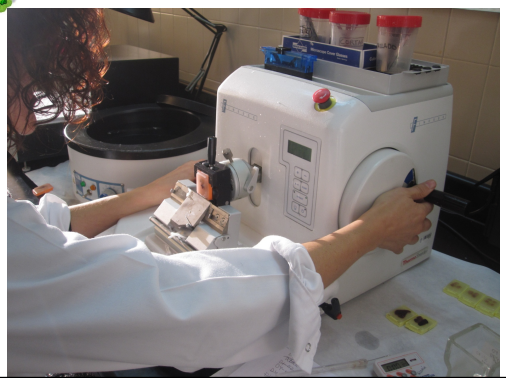
Necropsy



Histological procedure

Histological procedure

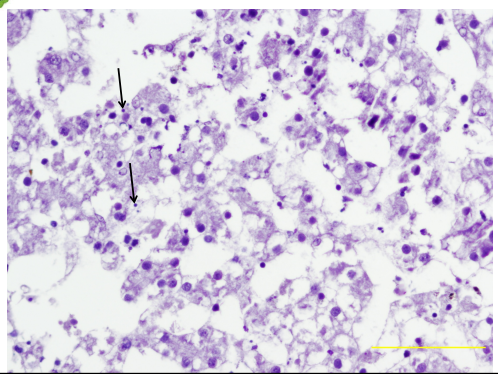
Histological procedure



Histological procedure

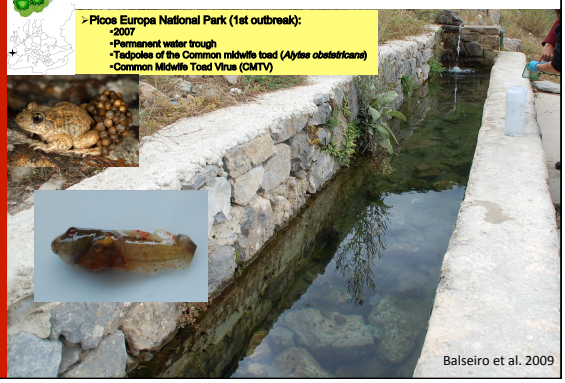
Histological procedure

Histological procedure



Mass mortality in Spain

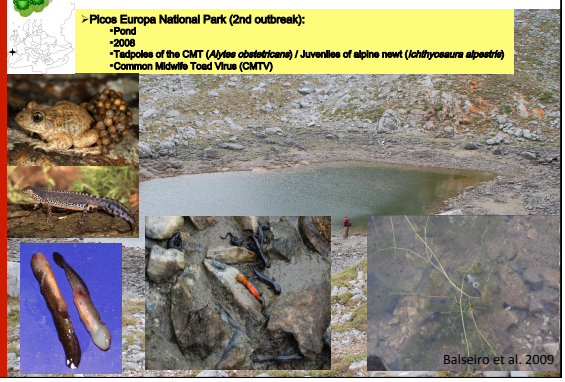
- Picos Europa National Park (1st outbreak):
  - 2007
  - Permanent water trough
  - Tadpoles of the Common midwife toad (*Alytes obstetricans*)
  - Common Midwife Toad Virus (CMTV)



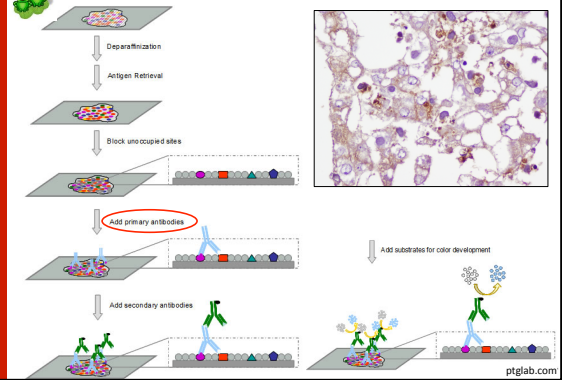
Balseiro et al. 2009

Mass mortality in Spain

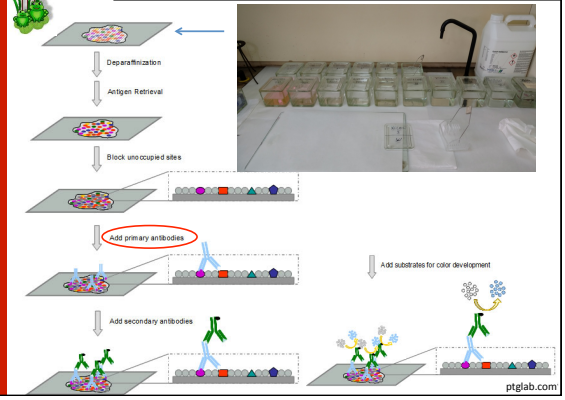
- Picos Europa National Park (2nd outbreak):
  - Pond
  - 2006
  - Tadpoles of the CMT (*Alytes obstetricans*) / Juveniles of alpine newt (*Ichthyosaura alpestris*)
  - Common Midwife Toad Virus (CMTV)



Immunohistochemistry

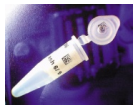


Immunohistochemistry



Immunohistochemistry

Available primary antibodies



➢**OIE: World Organisation for Animal Health (Dr. Whittington)**

Chapter 2.1.2. Infection with Ranavirus

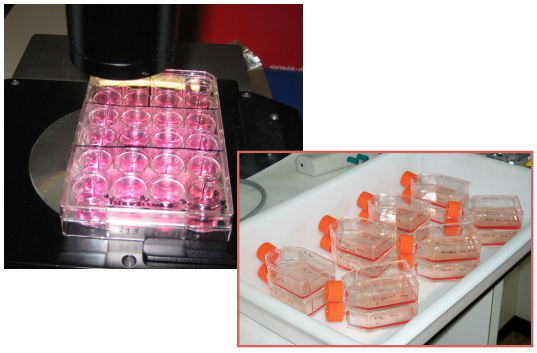
http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/2.1.02_RANAVIRUS.pdf

-Anti-Epizootic haematopoietic necrosis virus (EHNV) antibody-

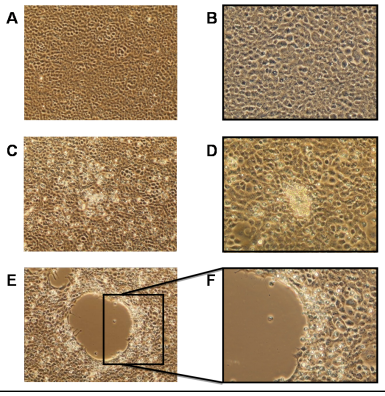
➢**SERIDA (Dr. Ana Balseiro):**

-Anti-Common Midwife Toad Virus (CMTV)-

Virus culture



Virus culture



Ranavirus purification

Cell lised by 3 freeze-thawing cycles

↓

Sonicated 4 min

↓ Centrifugated and resuspended

Pellet purified layered onto a 30% sucrose cushion

↓ Centrifugated 38000 rpm 60 min 4ºC

Pellet resuspended in PBS and ready to use

Anti-Ranavirus-primary antibody

➢ **Purified virions (175 µg/inoculation)**

Resuspended in PBS

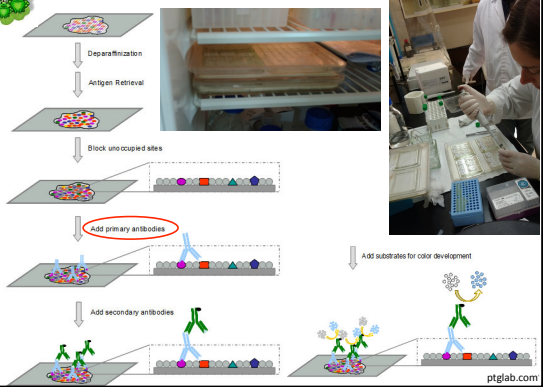
Rabbit immunization

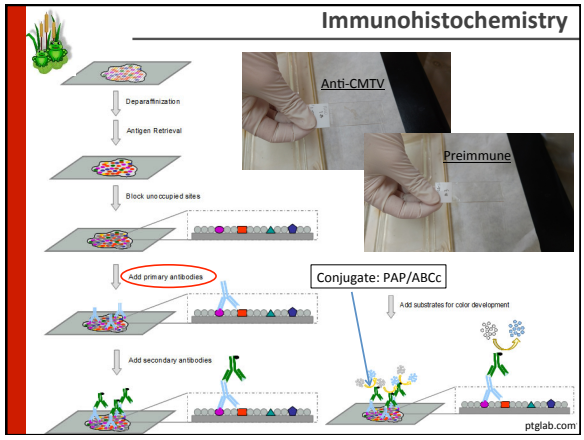
- **4 intramuscle injections with Freund´s adjuvant during 4 weeks**
- **1 intravenous injection without Freund´s adjuvant the 5th week**

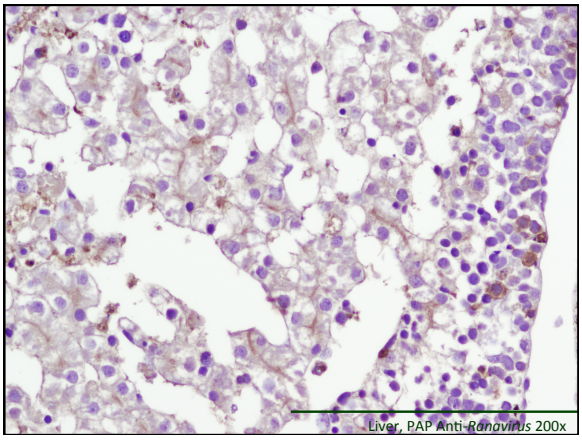
5 days

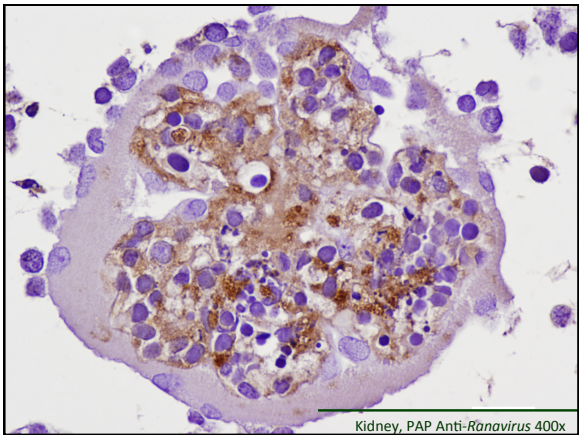
Primary antibody

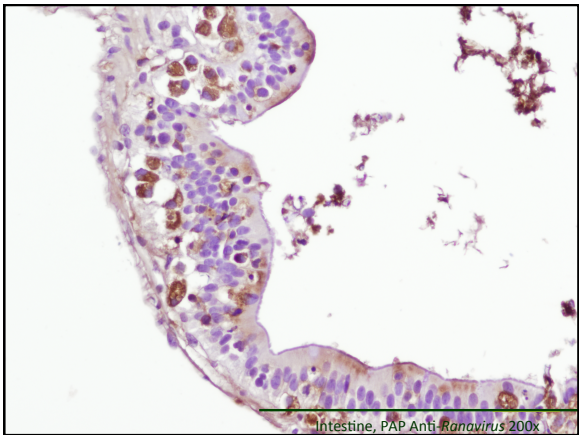
Immunohistochemistry



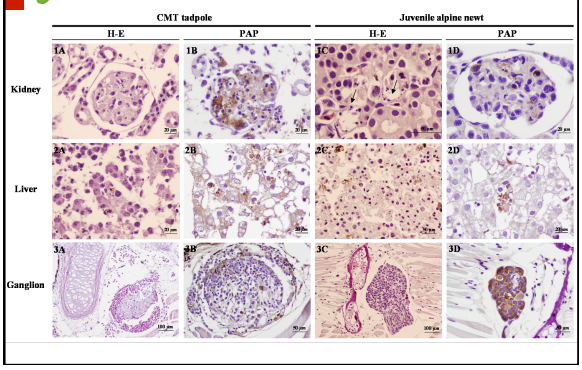



Liver, PAP Anti-*Ranavirus* 200x


Kidney, PAP Anti-*Ranavirus* 400x



Immunohistochemistry



Diagnostic techniques

Any question about IHC?

In situ hybridization

OBJECTIVE

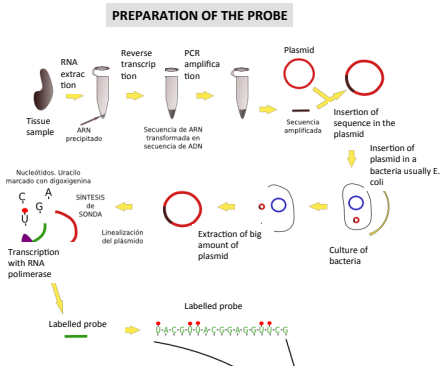
Localize a specific sequence of DNA in tissues

In situ hybridization

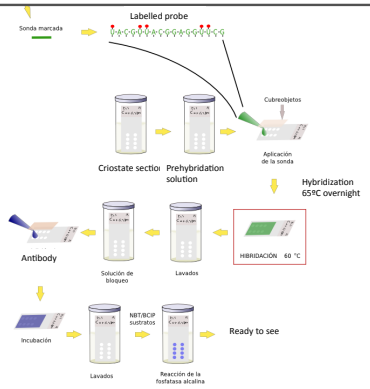
Tissue fixation

-Formalin 4-6 hours
-Glutaraldehyde
-Paraformaldehyde

In situ hybridization



In situ hybridization



In situ hybridization

Characterization of Chinese giant salamander iridovirus tissue tropism and inflammatory response after infection (Jiang et al. 2015)

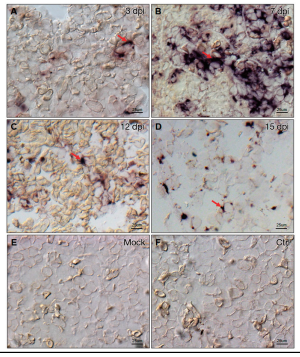
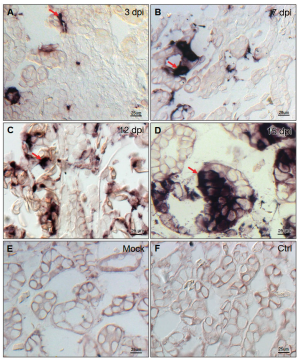


Fig. 1. ***Andrias davidiamus***. *In situ hybridization with a digoxi* - genin (DIG)-labeled RNA probe specific to the major capsid protein (MCP) in the **spleen** sections obtained from Chinese giant salamander iridovirus (GSIV)-infected salamanders. (A–D) Spleen sections sampled at 3, 7, 12 and 15 d post-infection (dpi). (E) Mock-infected spleen sections. (F) Ctrl: 15 dpi spleen stained with the secondary anti-DIG antibody alone. Arrows indicate a positive signal. Scale bars = 25 µm

In situ hybridization

Fig. 1. *Andrias davidiamus. In situ* **Kidney** (A–D) Spleen sections sampled at 3, 7, 12 and 15 d post-infection (dpi). (E) Mock-infected kidney sections. (F) Ctrl: 15 dpi kidney stained with the secondary anti-DIG antibody alone. Arrows indicate a positive signal. Scale bars = 25 µm



In situ hybridization

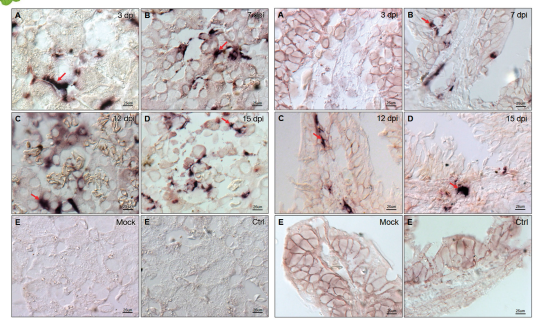


Fig. 1. *Andrias davidianus. In situ* **Liver and gut** (A–D) Tissues sections sampled at 3, 7, 12 and 15 d post-infection (dpi). (E) Mock-infected tissues sections. (F) Ctrl: 15 dpi tissues stained with the secondary anti-DIG antibody alone. Arrows indicate a positive signal. Scale bars = 25 µm

Diagnostic techniques

Any question about *in situ* hybridization?

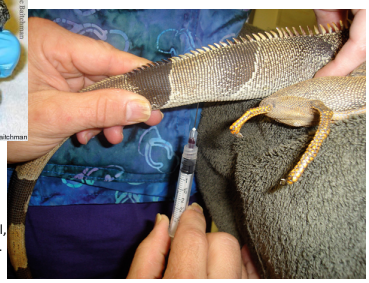
ELISA

OBJECTIVE

Detect antibodies or antigen in serum

How to obtain serum

Blood sampling
Ventral abdominal vein
Small gauge needle



Collecting blood from the ventral tail vein of a green iguana. Most patients tolerate this procedure well, with only minimal restraint. (Douglas Mader)

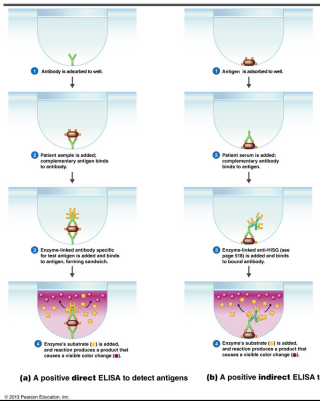
How to obtain serum

Lafeber.com

ELISA

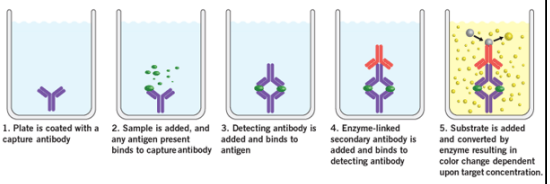
96 well plates

ELISA



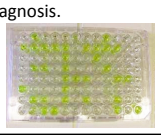
ELISA

Sandwich ELISA



ELISA

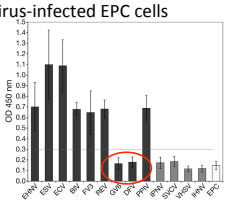
- **Simple method** for the detection and identification of viral pathogens.
- Important **tool** that assist in reducing losses from disease outbreaks and in preventing the spread of viral diseases by trade in live aquatic animals/products.
- Thanks to its sensitivity and specificity, **rapid** processing of **large numbers** of samples, **low cost**, reagent stability, and **ease** of procedure, serology could be very helpful in screening some fish populations but it has not yet been validated for routine diagnosis.

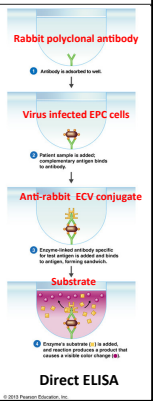


ELISA

Evaluation of a polyclonal antibody for the detection and identification of ranaviruses from freshwater fish and amphibians
Diseases of Aquatic Organisms, Cinkova et al. 2010

- A rabbit polyclonal antibody (PAb) raised against European catfish virus from infected EPC cells
- Direct ELISA
- Samples: virus-infected EPC cells



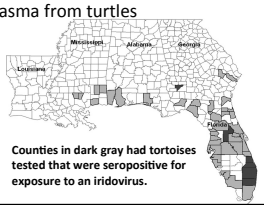


Direct ELISA

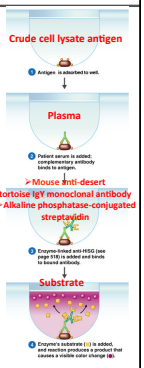
ELISA

Development and use of an indirect enzyme-linked immunosorbent assay for detection of iridovirus exposure in gopher tortoises (*Gopherus polyphemus*) and eastern box turtles (*Terrapene carolina carolina*)
Veterinary Microbiology, Johnson et al. 2010

- Burmese star tortoises Ranavirus (BSTRV) was used as antigen
- Indirect ELISA
- Samples: plasma from turtles



Counties in dark gray had tortoises tested that were seropositive for exposure to an iridovirus.

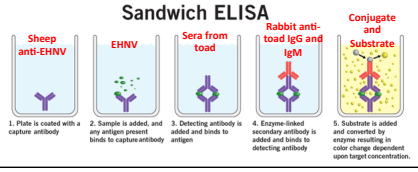


Indirect ELISA

ELISA

An improved enzyme linked immunosorbent assay for detection of anti-ranavirus antibodies in the serum of the giant toad (*Bufo marinus)*
Developmental and Comparative Immunology, Zupanovic et al. 1998

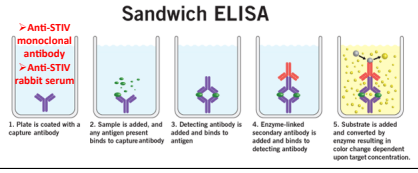
- EHNV was used as the antigen
- Sandwich ELISA
- Samples: sera from field and challanged toads



ELISA

A double antibody sandwich enzyme-linked immunosorbent assay for detection of soft-shelled turtle iridovirus antigens
Journal of Virological Methods, Zhang et al. 2010

- Two antibodies
- Sandwich ELISA
- Samples: sera from turtles



Diagnostic techniques

Any question about ELISA?

IHC vs. *in situ* hybridization vs. ELISA

IHC

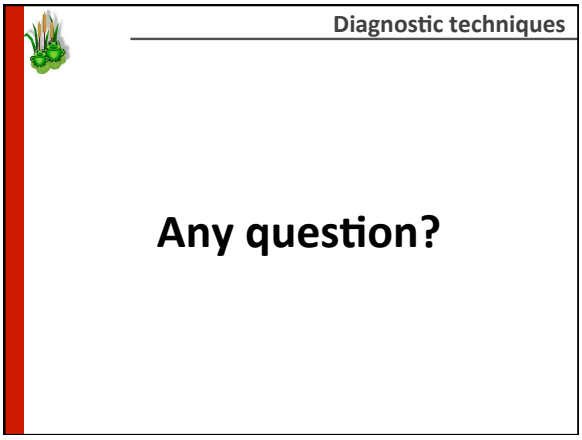
- **Advantages: pathogeny (Target organs, tissue distribution) and pathology**
- **Disadvantages: antibody and technology, more time, more expensive, dead animal**

In situ hybridization

- **Advantages: detect low DNA virus, disease pathogenesis**
- **Disadvantages: technology, precise optimization and expensive; different probe for each species; dead animal**

ELISA

- **Advantages: Fast and cheap; population status*; in vivo***
- **Disadvantages: individual immune response; only confirms infection, not disease; sensitivity and specificity**


Diagnostic techniques
Any question?