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levenslang gezond

LABORATORY TECHNICAL INFORMATION ON LSD

Serology

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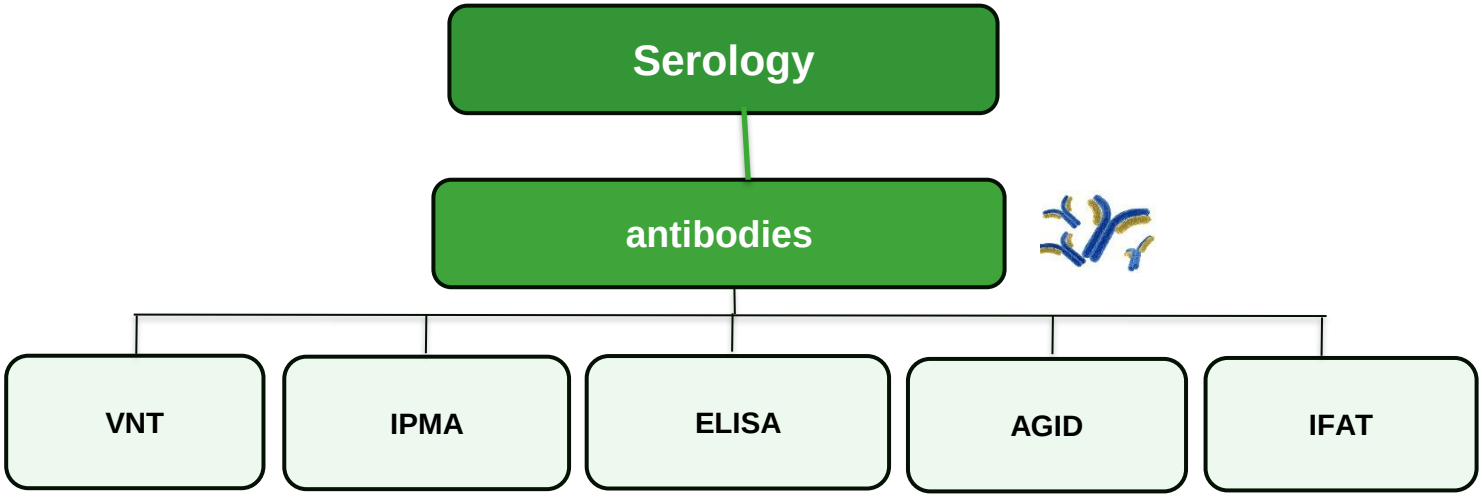
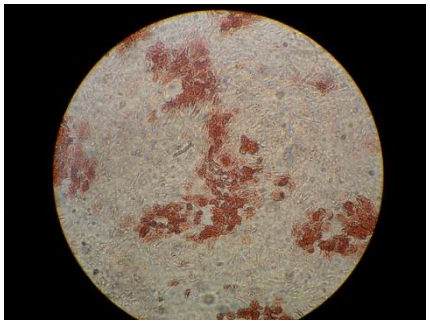
Laboratory diagnosis - serology





Serology

1. Virus neutralization test (VNT)

- **Gold standard**
- **Two formats**

  ✓ **Constant titer of capripoxvirus (100 $TCID_{50}$) Vs dilution test sera**
  → **expressed as an antibody titer (at Sciensano: > 1/50 as positive)**

  ✓ **Constant serum dilution Vs different virus dilution**
  → **Comparison test sample with a negative reference**
  → **Expressed as neutralisation index (cut-off NI of>= 1,5)**

- **Detection with CPE Vs immunoperoxidase –staining (cfr Virology)**

Serology

1. Virus neutralization test (VNT)

- Detection of neutralising antibodies
- No special equipment

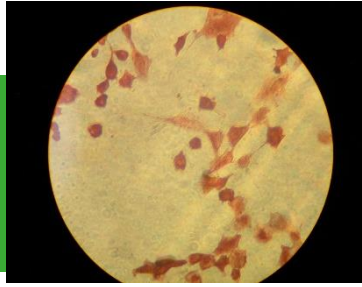
- Labor and time intensive
- Multiplication of virus possible → Biosafety !!
- NI-method: Negative reference?
- No high throughput

Serology

2. Immunoperoxidase monolayer assay (IPMA):

- Monolayer-like ELISA
- In brief:
  - ❖ OA3.Ts monolayer → infect → incubate → fixation → store
  - ❖ Test serum is potential provider for primary anti-LSDV antibody
  - ❖ Secondary anti-bovine antibody coupled with peroxidase
  - ❖ Coloring if anti-LSDV Ab's are present
- 96-well format
- 2 serum dilutions tested in duplicate → 14 samples per plate
- Has been validated LSDV (fit for purpose for SPPV)

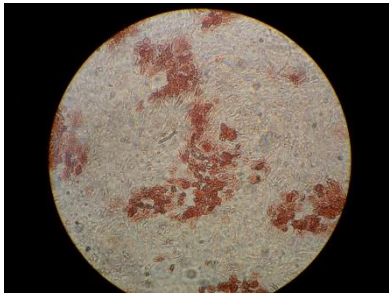
Serology



2. Immunoperoxidase monolayer assay (IPMA):

- Cost effective
- Highly Sensitive (earlier detection than ELISA)
- Once fixated, plates are safe → Can go out of BSL3
- No special equipment (light microscope, incubator)
- Semi- high throughput when plates are made in advance

- Labor and time consuming
  → However, plates can be prepared in advance → stabile
- Some training is required for interpretation
- Sensitive to sera quality



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Serology

Lumpy Skin Disease Virus (LSDV) / Capripoxvirus (CPV)

ID Screen® Capripox Double Antigen Multi-species

ELISA

Double Antigen ELISA for the detection of antibodies against capripoxviruses including lumpy skin disease virus (LSDV), sheeppox virus (SPPV) and goatpox virus (GTPV) in serum or plasma from cattle, sheep, goats or other susceptible species.

3. Commercial Elisa

- Easy to use
- No special equipment (Elisa reader)
- Robust
- High throughput
- High sensitivity / Specificity (better than VNT)

- Sub-clinical infection can be missed
- Detects Ab's less early than IPMA (IgM?)

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4. Inhouse Elisa systems

A. Proteins

- P32 (whole or truncated)
  - ❖ Expressed in E. Coli (Carn et al 1994; Heine et al 1999, Ebrahimi-Jam et al 2021)
  - ❖ Expressed in Yeast (Bhanot et al 2009)
- A34
  - ❖ Expressed in E. Coli (Berguido et al 2022)

B. Whole virus, inactivated (Babiuk et al., 2009, Sthitmatee et al 2023)

C. Peptides (Tian et al ., 2010)

Serology

- High throughput

- The production of sufficiently high volume of high quality antigen → practicality, biosafety point (whole virus ELISA)
- Variable sensitivity and specificity has been reported
- Interlaboratory issues

Caution:

- Look careful at the validation data !!
  - Number of samples used
  - Type of samples (clean Vs field samples; positivity: strong Vs weak …)

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5. Alternative techniques

A. Agar gel immunodiffusion test (AGID)

- Cross-reaction with other pox-viruses such as parapox

B. Indirect immunofluorescent antibody test (IFAT)

- High sensitivity + Flexible (other dyes/microscopes)
- Cross-reaction with other viruses such as orf or bovine popular stomatis needs to be kept in mnd

C. Western Blot against P32

- Specific and sensitive
- Expertise and costly

Sampling

- What is the purpose ? Screening / surveillance

Serum

- Is suited for ELISA, virus neutralization, IPMA
- Easy to collect
- Can remain present for a prolonged period of time

- Developments during the course of the infection (+/- 2 weeks) → not suited for early detection

Sampling

2 different types of tubes:

- With clot activator
  - Advantage:
    - ✓ Better separation between red blood cells and serum
    - ✓ Less hemolysis (important for IPMA ~false negative results)

- Without clot activator
  - Advantage:
    - ✓ Less expensive