# BTSF ACADEMY

Organisation and implementation of training activities to strengthen understanding, implementation and enforcement of EU law in the area of Sanitary and Phytosanitary (SPS) standards in EU Member States and neighbouring non-EU countries

## BTSF Programme on Sheep pox and Goat pox

Andy Haegeman

Valencia, Spain 30/01-01/02 2024

# BTSF ACADEMY

## Sheep and Goat Pox: Diagnostic tools and training opportunities.

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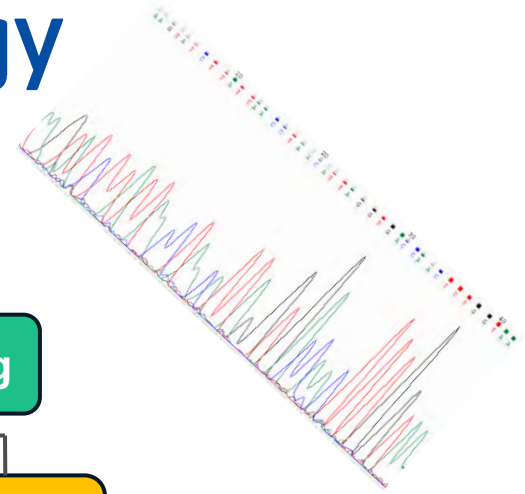
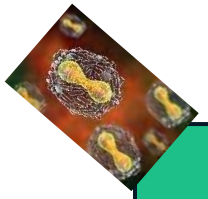
**BTSF Programme on Sheep pox and Goat pox**

Andy Haegeman, Nick De Regge

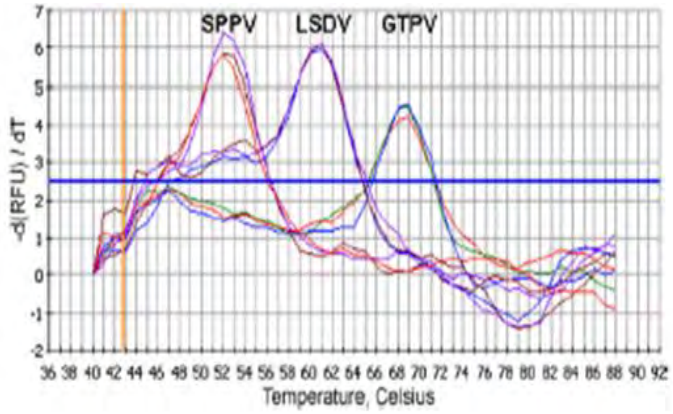
Valencia, Spain 30th January – 2nd February 2024

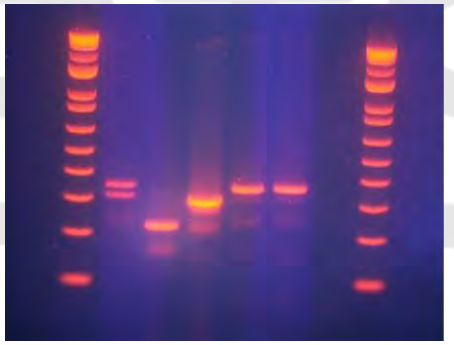
# BTSF Laboratory diagnosis - virology





- Virology
  - virus
    - Virus isolation
    - LFD
  - viral DNA
    - Pan capripox
    - Species specific PCR
    - DIVA PCR
    - Pen-side test
  - sequencing
    - Partial genome sequencing
    - WGS



| |
|---|
| 1kb ladder |
| 1. Lumpivax vaccine |
| 2. SPPV RM65 vaccine |
| 3. Field type SPPV |
| 4. Field type LSDV |
| 5. LSDV vaccine (Neehtling) |
| 6 Neg |
| 1kb ladder |

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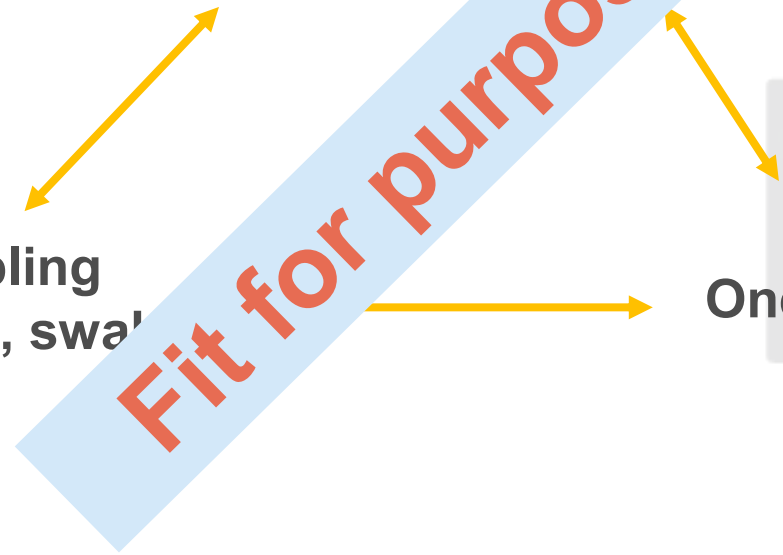
# BTSF Virology

Which test to use
??????

Why performed ?? Diagnosis, Research ing, First Suspicion….
Local situation?? Endem Outbreak….
Available resources?? Financia cal (Equipment, BSL?....)




Sampling
Sera, Blood, swa

One sample, Ten, hundreds….

# BTSF Virology: Virus isolation

## 1. Virus isolation

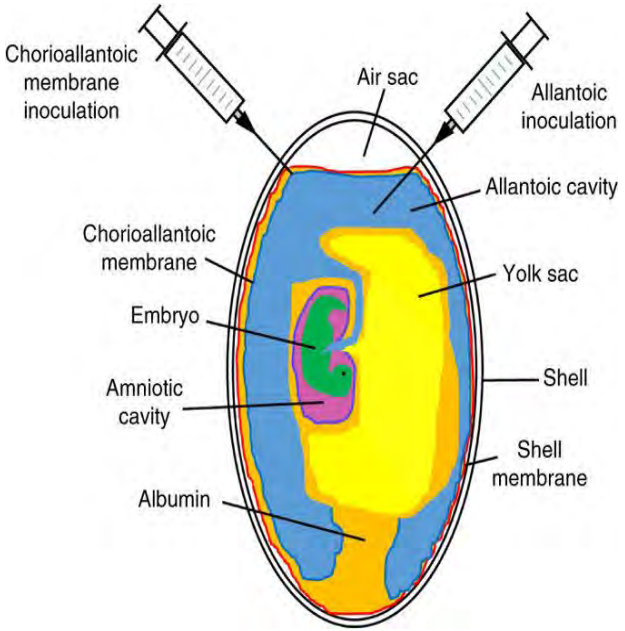
- Proof of presence of live virus → Risk analysis
- Obtaining isolate → further characterisation (Phylogeny, FGS…)
- Standard equipment (incubators…)

- Detection limit → timepoints of sampling
  → No suited for early detection or screening
- Virus multiplication → BSL3 needed + contamination !!!!
  → cleaning SOPs !!
- Time and labour intensive
  (multiple passages are sometime needed)

# BTSF Virology: virus isolation

- **A. Embryonated Chicken Eggs (ECE)**

## Chorio-Allantoic Membrane (CAM)




(Andersson et al; 2015)

- **High viral loads can be obtained**
- **Training and experience is required !**



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# BTSF Virology: virus isolation

- **B. Cell line**

## B.1 Primary cell lines (eg. Fetal lamb kidney or testis)

- Highly (highest ?) sensitive

- Limited number of passages
- Homogeneity

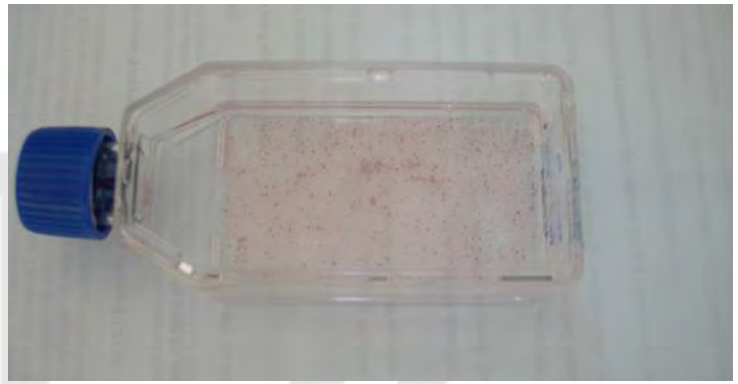
## B.2 Secondary cell lines

- Highly sensitive
- More passage are possible
- Large variety of cell lines available
  - ❖ OA3.Ts, MDBK, VERO, BHK21, AVK 58 …..

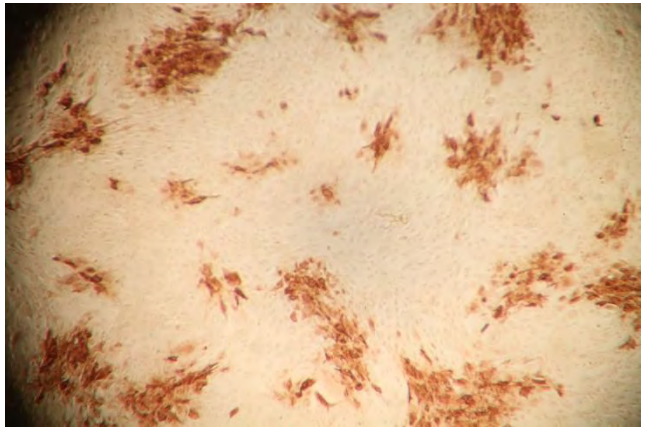
# BTSF Virology: virus isolation

## B.3.1 Cytopathogenic effect (CPE)

- ➕ Simple
- ➖ Not always (easily) detected (cfr cell line use)
  Not virus specific !
  - ❖ Lab contamination with other virus
  - ❖ Unknown pathogens in sample



## B.3.2 Immunoperoxidase-based staining



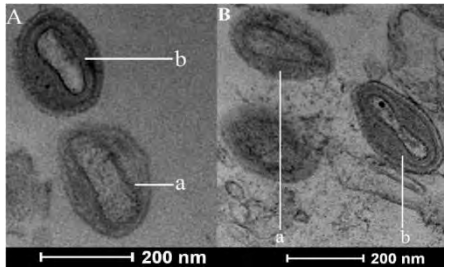
- ➕ Increased specificity and sensitivity
- ➖ Additional manipulation

# BTSF Virology: Virus Detection

## A. Electron Microscopy (EM)

- Very Fast

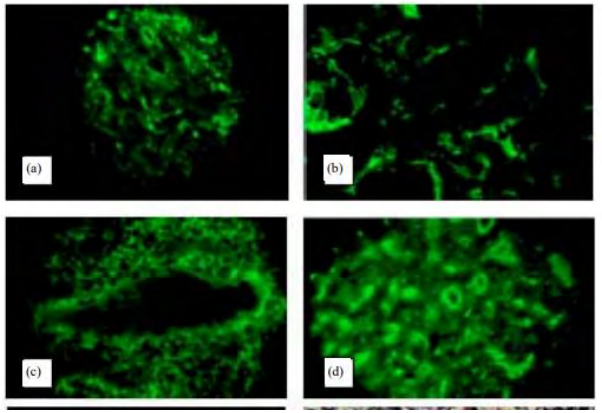
- Low sensitivity
- Special and expensive equipment
- No differentiation between Capripox genus
- No differentiation with Orthopox virus




## B. Direct immoperoxidase of immo-fluorescence methods

- Highly specific

- Equipment
- Low sensitivity




A.A. El-Kenawy and M.S. El-Tholoth, 2011; 10.3923/ijv.2011.158.166

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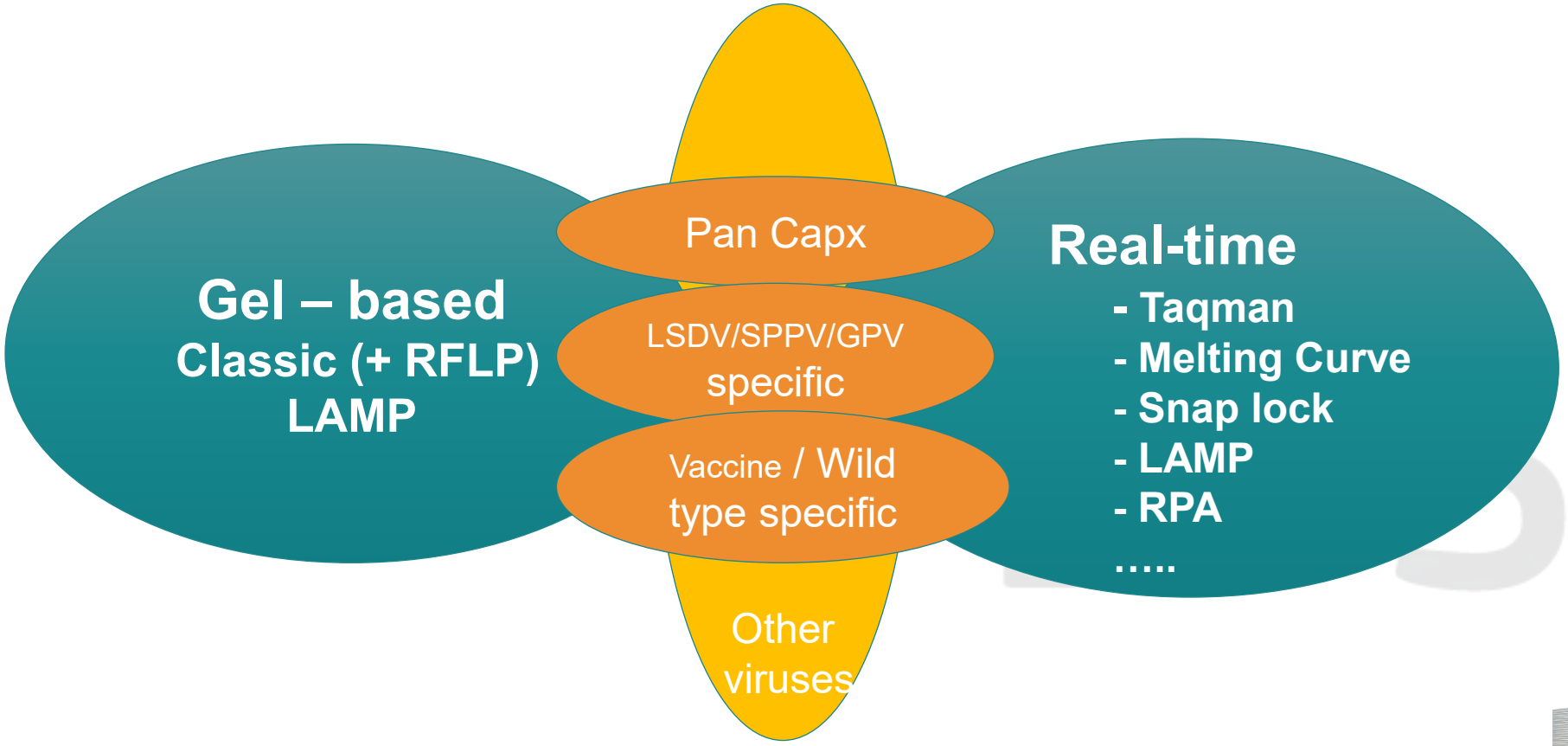
# BTSF Virology: virus genome detection

- **A variety of PCR methods have been developed (validated or not)**



# BTSF Virology: virus genome detection

? ? ? ? ? ?

## Which PCR to USE

# BTSF Virology: virus genome detection

## Fit for purpose !

- Do I need high sensitivity ?
- What are the financial implications
- Is the tech capacity and trained personal available
- Can I get the necessary materials in time
  → Example: during the Covid crisis: RT plates, pipet tips often delayed
- Multi-disease screening preferred?
- Is wild Vs vaccine type differentiation needed? → DIVA PCRs
- Do I need differentiation within capripox desired or is a pan-Capripox PCR sufficient??
- Validation needed? And to what extent?
- ……

- First case, suspicion, import: YES
- Epidemic screening or large scale screening : No
- I will be testing mainly lesions and scabs: No
- I will be testing blood, swabs: Yes
- ……

# BTSF Virology: virus genome detection

**Pan Capx**

**Real-time**
**Taqman (hydrolysis)**

Bowden et al., 2008
Haegeman et al 2013 (panel)
Balinsky et al 2008
ID Gene™ Capripox Virus Triplex (commercial)

**Multi-disease**

**Real-time**

He et al 2017


**SPPV Vs GTPV**

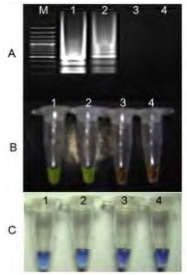
**Real-time**
**LAMP assay**

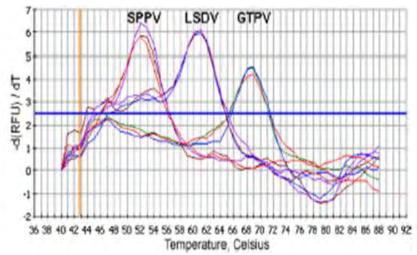
Venkatesan et al 2016 (LAMP)
Wolf et al 2021 (Taqman)
Lamien et al 2011 (melting curves)

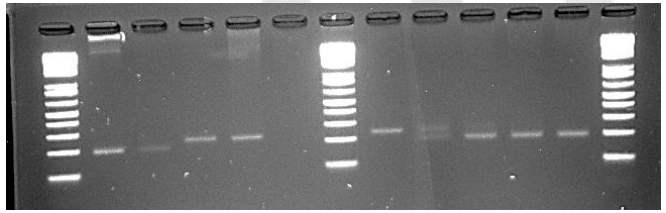
**SPPV: RM65 Vs wild type**

**Gel-based**

Haegeman et al 2015



Has been adapted for real-time detection using LC Green or in a Taqman version

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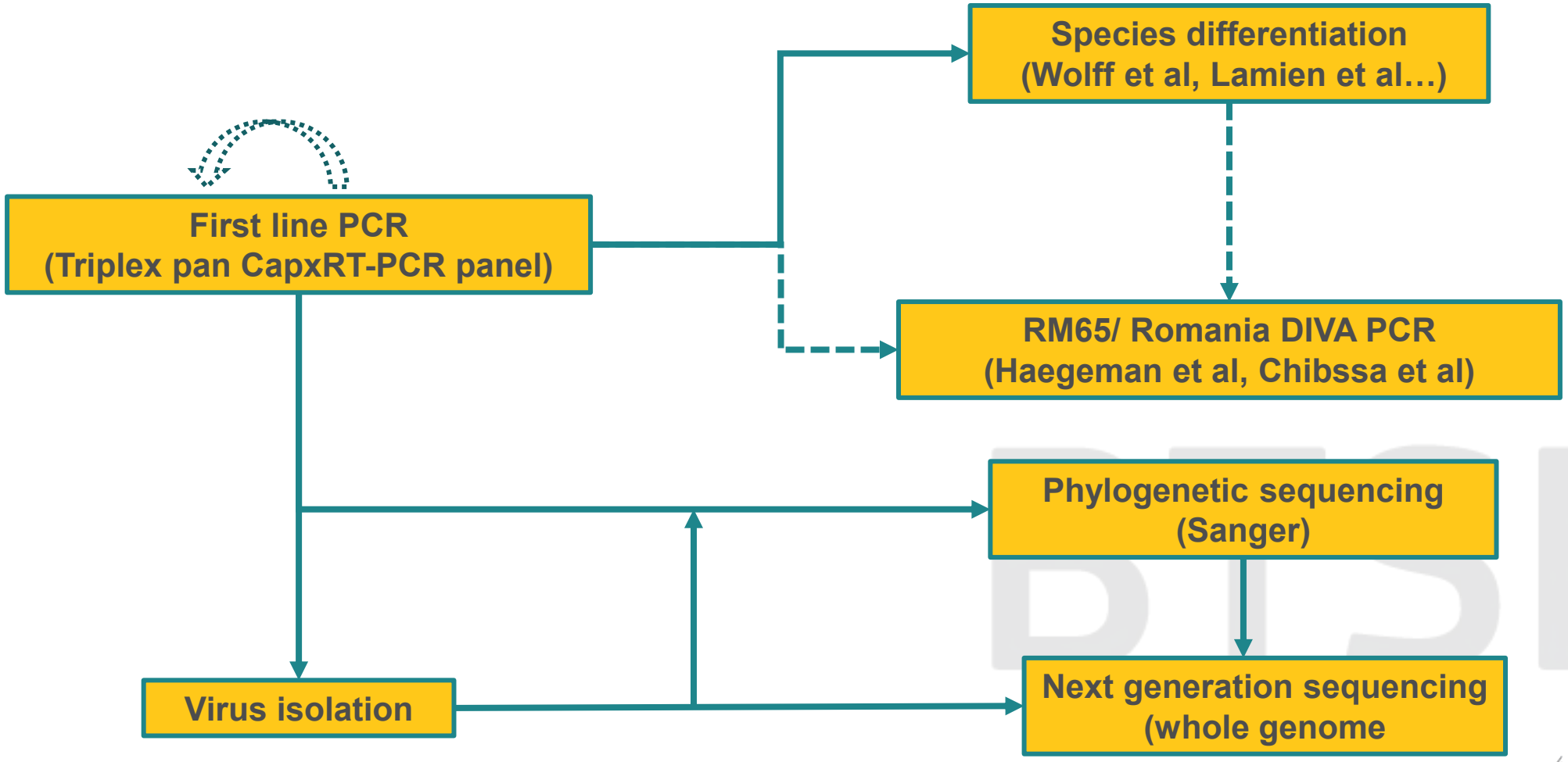
# BTSF Virology: PCR workflow Sciensano



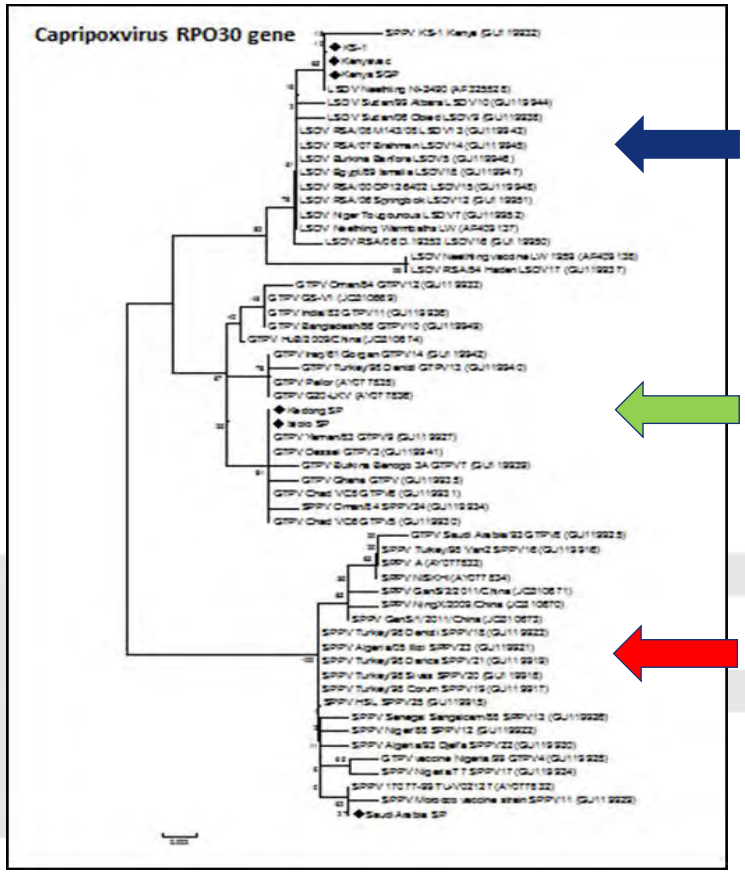
# BTSF Virology: Sequencing and phylogenetics

## A. Partial sequences

- RPO30, GPCR, EEV….

## B. Complete genome sequences

- PACBio, Illumina, Nanopore….




- Not expensive by commercial companies

- Lot of material needed with high viral load
- Data analysing requires highly specific training
- Less suitable for routine screening in many laboratories

→ However, Future??? Quicker, pipelines…..

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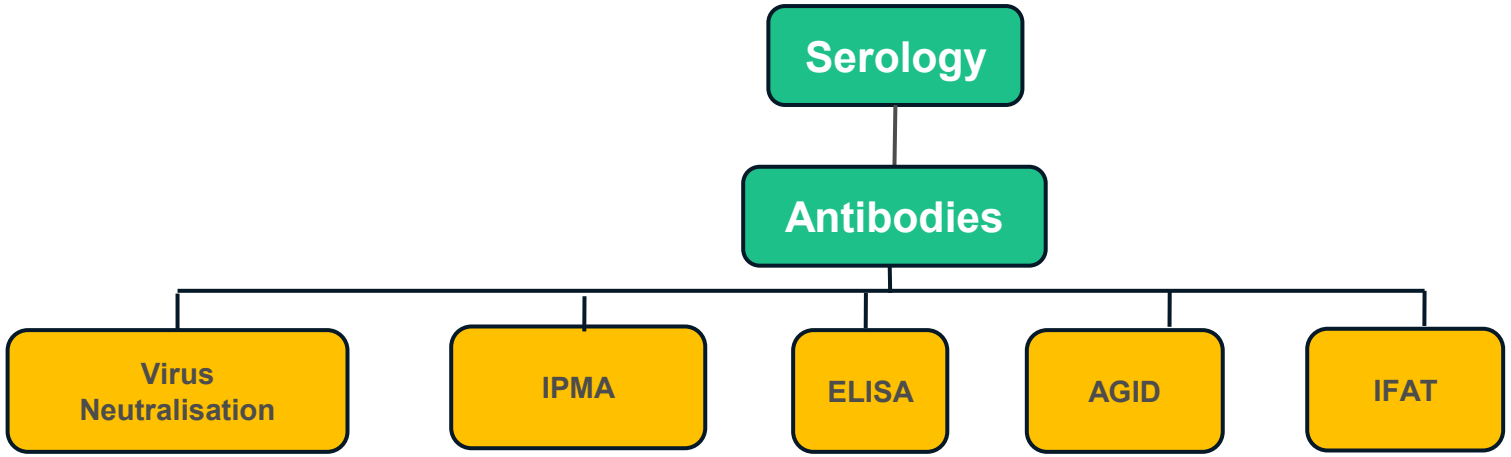
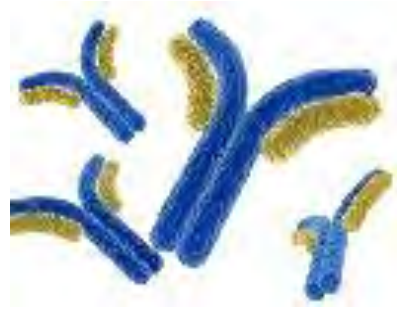
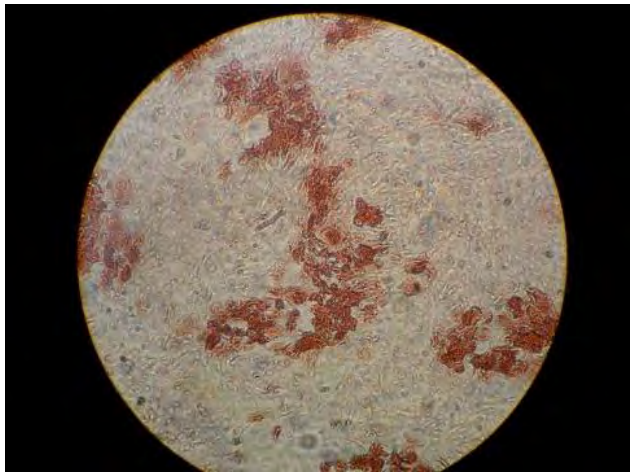
# BTSF Laboratory diagnosis - Serology





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# BTSF Serology: Virus Neutralisation Test (VNT)

## 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)

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

➕ Detection of neutralising antibodies
No special equipment

# BTSF Serology

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No special equipment

# BTSF Serology

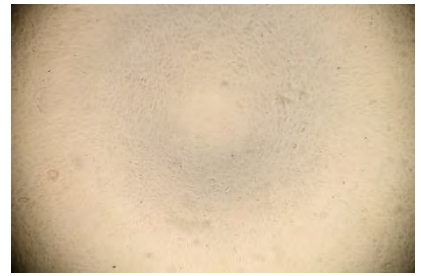
## 2. Immunoperoxidase monolayer assay (IPMA):

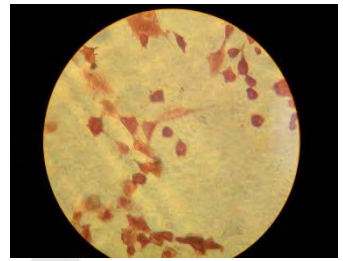
- Monolayer-like ELISA

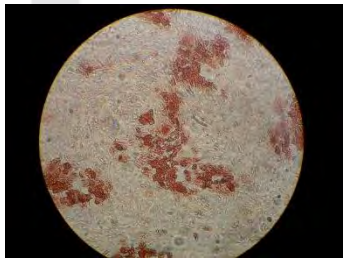
  In brief:

  - ❖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)









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# BTSF Serology: IPMA

## 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

# BTSF Serology

## 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?)

# BTSF Serology

## 4. Inhouse ELISA

### 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)

# BTSF Serology: Inhouse ELISA

## 4. Inhouse ELISA

- 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 …)

# BTSF Serology: Inhouse ELISA

## 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

# BTSF Serology: Overview

| Test | Advantages | Disadvantages |
|---|---|---|
| IFAT | - High sensitivity<br>- Flexible (other dyes/microscopes) | - Cross reaction<br>- Time and costs |
| VNT | - Reliable (gold standard)<br>- 2 methods | - Live virus<br>- Time and costs<br>- CPE effect ! Not virus specific |
| Western Blot | - High specific<br>- High sensitive | - Difficult<br>- Expensive |
| IPMA | - Early detection<br>- Fast results if plates prepared<br>- Highly sensitivity | -Experience<br>- Live virus<br>- More sensitive to sample quality |
| ELISA | - Commercially available<br>- Easy to do<br>- High throughput<br>- Good sensitivity / specificity | - Not for early detection (IgM??) |

# BTSF Training

**Sciensano (Belgium): European Union Reference Laboratory for Capripox**

*** Proficiency testing (yearly)**

*** Molecular and serological diagnostics**
- **Real-time and gel-based PCR (panCapx, DIVA….)**
- **IPMA and VNT**

*** Virus isolation (cell culture)**

*** Sequencing**

# BTSF ACADEMY

## Thank you!


**European Commission**
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