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levenslang gezond

LABORATORY TECHNICAL INFORMATION ON LSD

Virology

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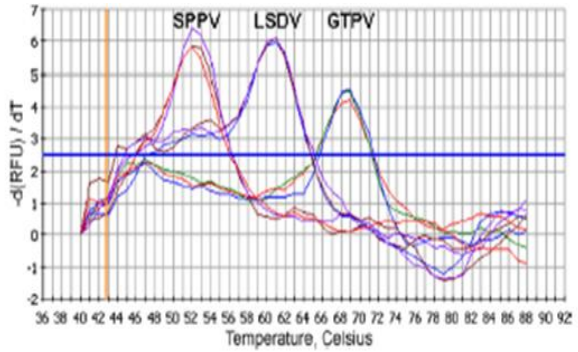
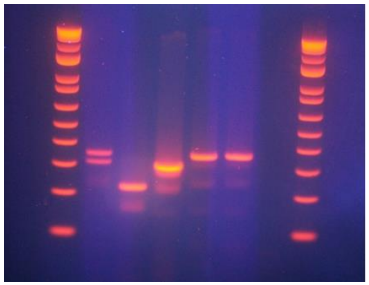
Laboratory diagnosis - virology

- **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

Virology

Which test to use ??????

Why performed ?? Diagnosis, Research, Screening, First Suspicion….
Local situation?? Endemic, Free, Outbreak….
Available resources?? Financial, Practical (Equipment, BSL?....)
……

Fit for purpose !!

Sampling
Sera, Blood, swabs….

One sample, Ten, hundreds….

Virology: Virus isolation

1. Virus isolation

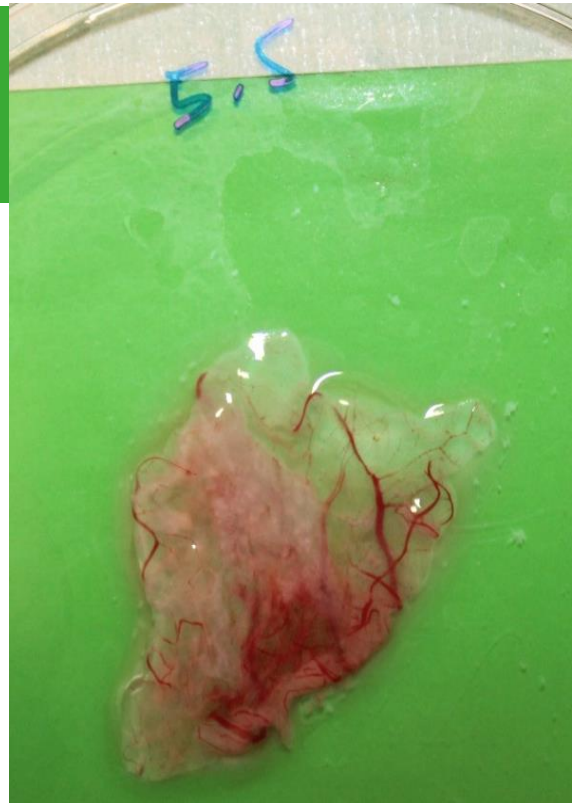
- Proof of presence of live virus → Risk analysis
- Obtaining isolate → further characterisation (Phylogeny, FGS…)
- Standard equipment (incubators…)

- 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)

Virology: Virus isolation

A. Embryonated Chicken Eggs (ECE)

- Chorio-Allantoic Membrane (CAM)



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

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

Virology: Virus isolation

B. Cell line

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

B.2 Secondary cell lines

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

Virology: Virus isolation

B. Cell line

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

B.2 Secondary cell lines

B.3 Detection

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**

Virology: Virus isolation

B. Cell line

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

B.2 Secondary cell lines

B.3 Detection

B.3.2 Immoperoxidase-based stainig

- Increased specificity and sensitivity
- Additional manipulation

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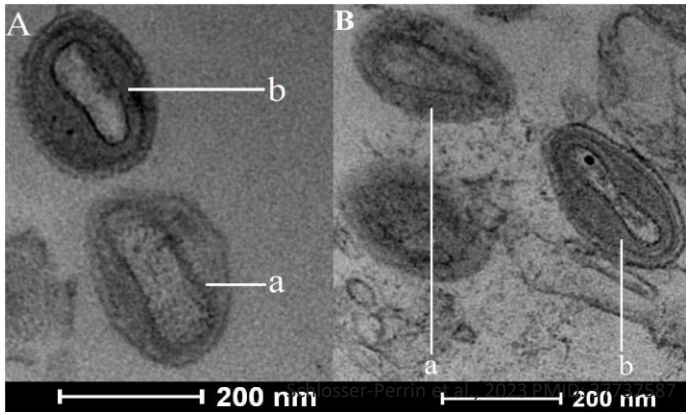
Virology: Virus Detection

2. Virus Detection

A. Electron Microscopy (EM)



- Very Fast

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

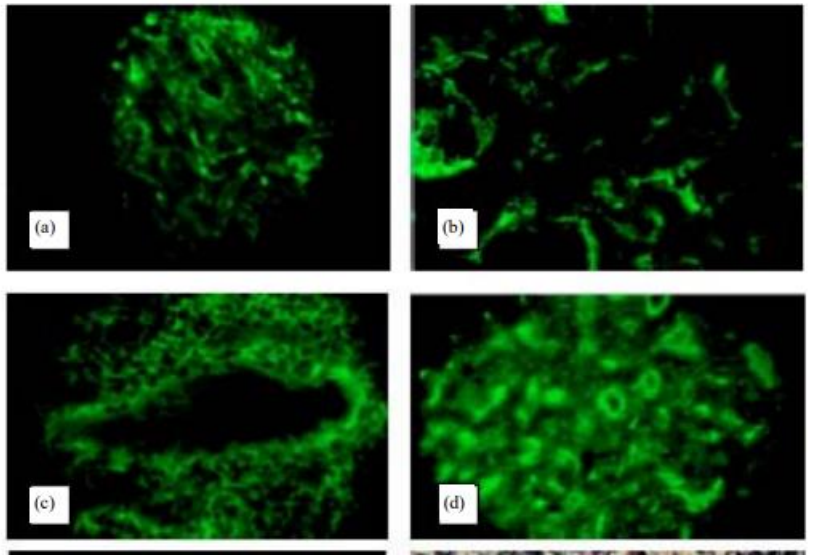
Virology: Virus Detection

2. Virus Detection

A. Electron Microscopy (EM)

B. Direct immoperoxidase of immofluorescence methods

- Highly specific
- Equipment
- Low sensitivity



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

Virology: Virus Detection

2. Virus Detection

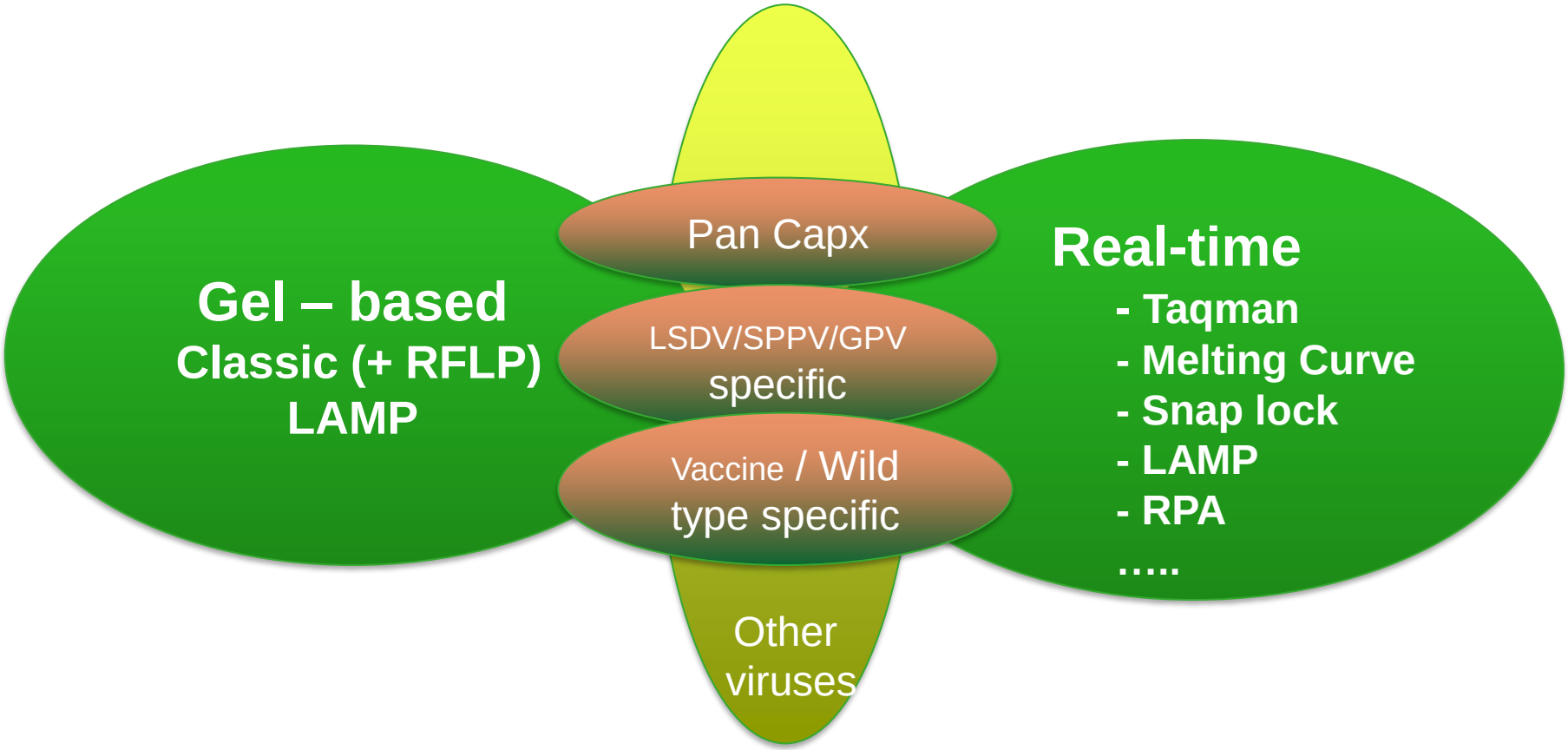
A. Electron Microscopy (EM)

B. Direct immoperoxidase of immofluorescence methods

C. Agar gel precipitation test (AGPT)

- Low sensitivity
- Cross reactivity parapox virus

Virology: Virus Genome Detection



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? ? ? ? ? ?

WHICH PCR TO USE

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Which PCR to use

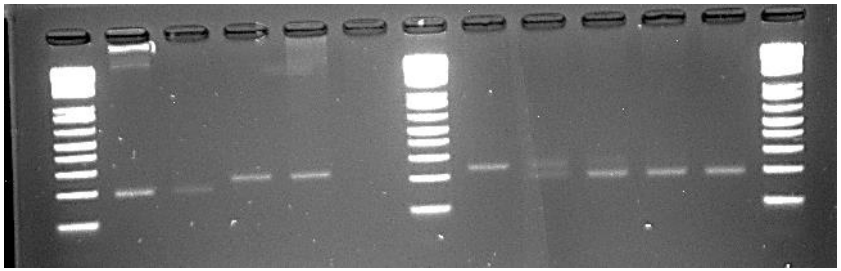
Unfortunately

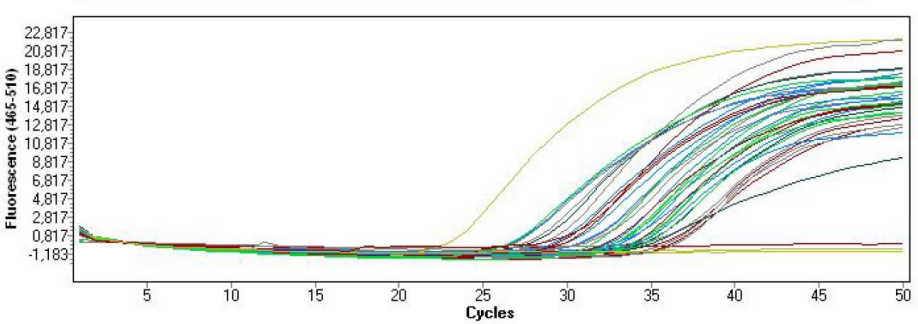
→ **NO** simple answer !!!!

BUT some things to consider

Which PCR to use

- PCR technique: Gel-based (GB) Versus Real-time (RT)
  - Sensitivity & Specificity: In general RT > GB
  - Speed: RT > GB
  - Lab equipment tech level: RT > GB
  - Reagent costs: RT > GB
  - Traceability: easier with RT
  - Ability of remote access with RT
  - Interpretation issues: RT ~= GB
  - Personal training RT > GB





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Which PCR to use

- PCR technique: Gel-based (GB) Versus Real-time (RT)
- Fit for purpose !
    - Do I need high sensitivity ?
        - First case, suspicion, import: YES
        - Epidemic screening or large scale screening : probably Not
        - I will be testing mainly lesions and scabs: No
        - I will be testing blood, swabs: Yes
        - ……
    - 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

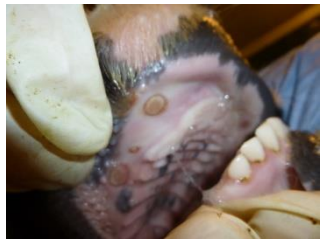
Virology and Sampling

- **What is the purpose ? Diagnosis**

- Wound crusts → Ideal to confirm clinical picture

  – Is suited for virus isolation and PCR due to high viral load and remains long time positive
  – Easy to collect
  – No need for the most sensitive test

  – Extra care is needed in sampling handling and analysis → High risk of contamination in the lab !! Extra cleaning is warranted ! Changing gloves also in the field !



- Nodule and skin lesion

  – Similar remarks but you need to take a biopsy !

  → Change biopsy needles after use !

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Sampling

- **What is the purpose ? Diagnosis**
  - EDTA Blood

  - Is suited for PCR (and virus isolation)
  - Easy to collect
  - Strength of viremia is more time dependent !

→ You can have nodules while the virus is cleared from the animal

→ Sensitivity is more important

  - Swabs (buccal, nasal…)
    - Same remarks as for blood

Sampling

- EDTA Blood
  - No pretreatment required
  - Examine blood quality ➡ tracing important, linked to PCR results ?
    - Non-coagulated
    - Fresh blood

- Important for collecting EDTA blood!
  - Blood tubes: check expiration date
  - Shaking after blood collection (EDTA mixed with blood)

Sampling

- Tissues, organs (cfr slaughterhouse)…
  - Cut small pieces and put in Eppendorf tube
  - Attention:

    - Large pieces are difficult to homogenize:
    - Membranes and connective tissues: hard to homogenize and digest
    - Fat residues can cause problems during subsequent seps