

CODA - CERVA

Update on diagnosis, vaccines and other aspects of Lumpy Skin Disease.

Annebel De Vleeschauwer, Céline Demarez, Andy Haegeman, Ilse De Leeuw, Laurent Mostin, Maria Vastag, Kris De Clercq

Assist EC and Countries

- Reply to (Official) Questions
- Technical input
 - Lab protocols for laboratories
- Missions and Trainings on the request of a country
 - CVET
 - TAIEX
 - BTSF (Better Training for Safer Food)
 - STM (Sustained Technical assistance Missions)

Support Countries for Diagnosis



- Training clinical signs
 - Austria
 - The Netherlands
 - Ireland
 - Germany
 - UK
 - OIE

- Training virological and serological laboratory techniques at CODA-CERVA
 - Bulgaria
 - Greece
 - Romania
 - Kosovo



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Update on Diagnostic techniques for Capripox viruses LSD - SPPX - GTPX

Commercial Pan Capx real-time PCR kits

- Commercial Kits:
 - Techne: with an internal control (IC)
 - Genesig® Standard Kit
 - Genesig® Advance Kit (LSDV116 RNA polymerase subunit), IC
 - Tetracore
 - Biosellal, IC



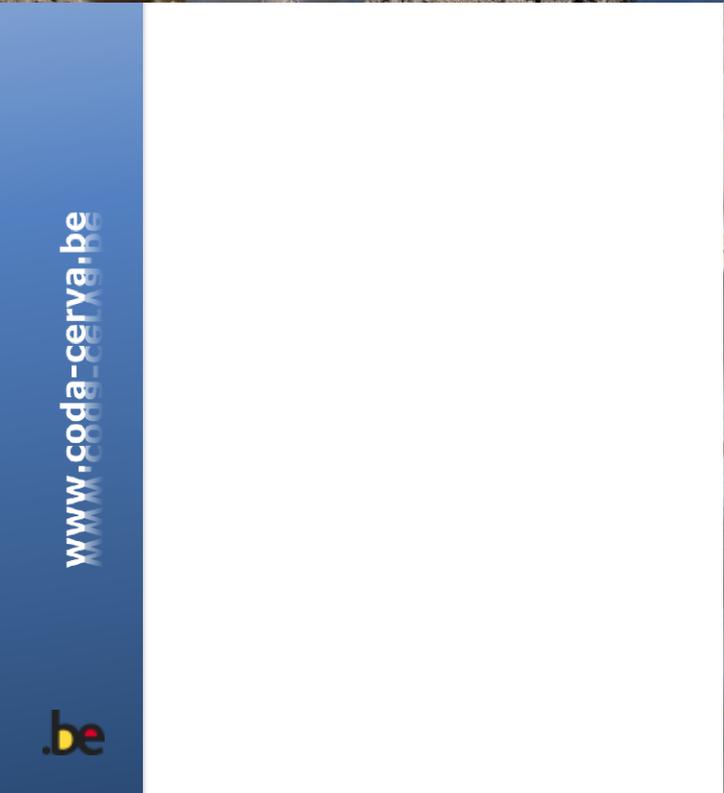
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DIVA



Gel-based PCR: differentiating wild type LSDV and vaccine strains

- Needed in case clinical signs are detected in vaccinated herds.

Journal of Virological Methods 199 (2014) 95–101



ELSEVIER

Contents lists available at [ScienceDirect](#)

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet



016)

Development of an assay to differentiate between virulent and vaccine strains of lumpy skin disease virus (LSDV)



Sophia Menasherow, Marisol Rubinstein-Giuni, Anita Kovtunenکو, Yevgeny Eyngor, Orly Fridgut, Ditza Rotenberg, Yevgeny Khinich, Yehuda Stram*

Veterinary Microbiology 201 (2017) 78–84



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Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic



Lumpy skin disease outbreaks in Greece during 2015–16, implementation of emergency immunization and genetic differentiation between field isolates and vaccine virus strains



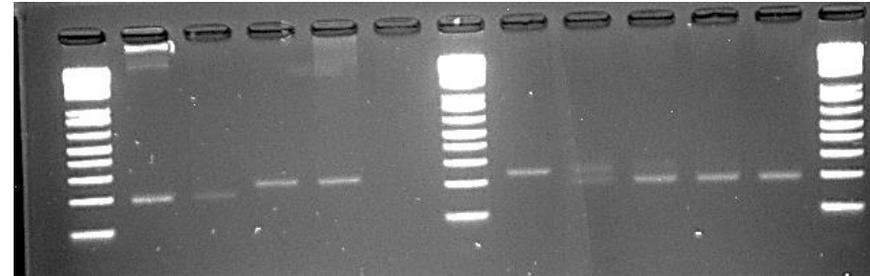
Eirini I. Agianniotaki^a, Konstantia E. Tasioudi^a, Serafeim C. Chaintoutis^b,

Gel-based PCR: differentiating wild type sheeppox virus and vaccine strains

- Needed in case clinical signs are detected in vaccinated herds.

- Haegeman *et al* 2015

- ✓ Wild SPPV Vs RM-65 vaccine
- ✓ RM-65 vaccine: deletions in ORF 26
- ✓ PCR 1 (primer situated in deletion):
 - Amplifies only wild type
 - Has been adapted for real-time detection using LC Green
- ✓ PCR2 (primer after deletions):
 - Different amplicon length (vaccine is 41 bp shorter)



Transboundary and Emerging Diseases

Transboundary and Emerging Diseases

ORIGINAL ARTICLE

Investigation of a Possible Link Between Vaccination and the 2010 Sheep Pox Epizootic in Morocco

A. Haegeman¹, K. Zro^{2,3}, D. Sammin⁴, F. Vandenbussche⁵, M. M. Ennaji² and K. De Clercq¹

Real-time PCR: differentiating wild type and vaccine strains

- Haegeman et al 2015
 - ✓ Using LC Green for differentiation SPPV wild type and RM-65 vaccine
- Menasherow et al., 2016
 - ✓ Method improved by High-resolution melting (HRM) assay for LSDV
- Vidanovic et al 2016
 - ✓ 2 assays KV-2 and FLI
 - ✓ Taqman based
 - ✓ 27 bp deletion in EEV
 - ✓ KV-2: 113 bp // FLI: 121 bp
 - ✓ Both more sensitive than Menasherow et al., 2014 nested PCR
 - ✓ Less sensitive than Bowden real-time
 - ✓ Based on absence of signal rather than specific vaccine signal → Needs to be used in parallel other real-time PCR for Pan Capx to exclude vaccine strain
- Agianniotaki et al 2016
 - ✓ Taqman based
 - ✓ Wild type and vaccine probe
 - ✓ Discussed in detail later

Journal of Virological Methods 249 (2017) 48–57



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Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet



Development and validation of a TaqMan probe-based real-time PCR method for the differentiation of wild type lumpy skin disease virus from vaccine virus strains

Eirini I. Agianniotaki^{a,b}, Serafeim C. Chaintoutis^a, Andy Haegeman^c, Konstantia E. Tasioudi^b, Ilse De Leeuw^c, Panagiotis-Dimitrios Katsoulos^d, Achilleas Sachpatzidis^e, Kris De Clercq^c, Thomas Alexandropoulos^f, Zoe S. Polizopoulou^a, Eleni D. Chondrokouki^b, Chrysostomos I. Dovas^{a,*}

Characterisation of LSDV

○ Collaborations with Laboratories

- Israel
- Bulgaria
- Greece



AMERICAN
SOCIETY FOR
MICROBIOLOGY

genomeAnnouncements™

Complete Genome Sequence of the Lumpy Skin Disease Virus Isolated from the First Reported Case in Greece in 2015

Eirini I. Agianniotaki,^a Elisabeth Mathijs,^b Frank Vandenbussche,^b
Konstantia E. Tasioudi,^a Andy Haegeman,^c Peristera Iliadou,^a
 Serafeim C. Chaintoutis,^d Chrysostomos I. Dovas,^d Steven Van Borm,^b
Eleni D. Chondrokouki,^a Kris De Clercq^e

- Serbia

○ Bank of LSDV isolates

○ Assistance/Training in biosafe sample transport and regulations

Serology

Commercial ELISA

- Evaluation of the performance of a novel ELISA (ID-Vet) has been carried out using a large number of serum samples
- Detects antibodies approximately five months post-vaccination
- Performs well on herd/flock level
- Sensitivity is clearly better than VNT
- Individual animals with low antibody levels may not be detected: animals in early stage of infection; with mild disease; some vaccinated animals with low response

ID.vet
Innovative Diagnostics



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The importance of Virological and Serological Diagnostics

The importance of Virological and Serological Diagnostics

- 1) Under the new OIE rules virological and serological testing are part of the procedures for a country's recognition or recovery of LSD freedom !
- 2) The use of LSD vaccination generates the need for DIVA testing

OIE CHAPTER 11.11.
INFECTION WITH LUMPY SKIN DISEASE VIRUS



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Proficiency Testing

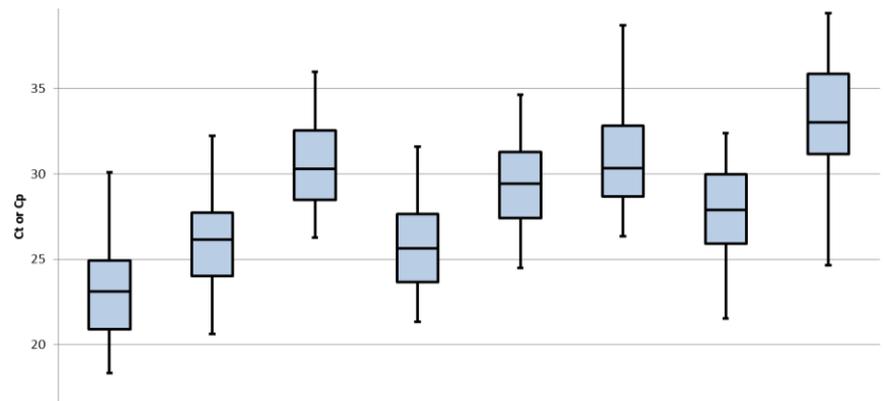
PROFICIENCY TESTING 2017

CAPRIPOX VIRUS (CAPX)

Detection of specific antibodies to capripox viruses in serum
and/or

Detection of capripox virus nucleic acid in cell culture supernatant
and tissue homogenate.

**Organised conform
ISO 17043**

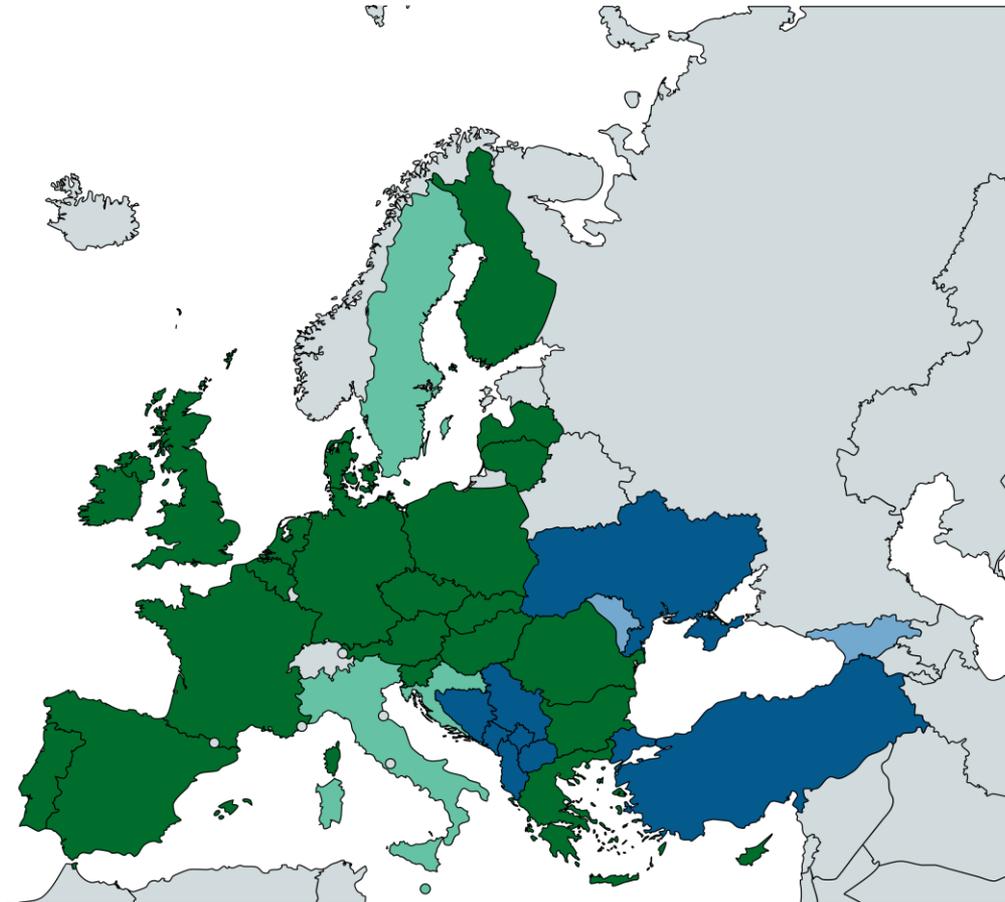


Participating Countries 2017



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- EU member state
- Non-Eu state
- EU member state participated
- Non-EU member state participated



PT Samples sent to

- **31 NRL**
 - 22 EU
 - 9 non-EU
- **14 Serology + Virology component**
 - 11 EU
 - 3 non-EU
- **16 Virology component only**
 - 11 EU
 - 6 non-EU

Created with mapchart.net ©

PT 2017 Conclusions

- All laboratories that participated to the serology component of the PT can successfully identify the absence or presence of antibodies to Capripox viruses in serum of bovine origin
- All but one laboratory that participated to the virology component of the PT can successfully detect capripox virus nucleic acid in samples
- In general most participating laboratories are able to discriminate between LSDV field and vaccine strain as well as to identify the different capripox virus species



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Annual Meeting NRLs

COMBINED PESTE DES PETITS RUMINANTS / CAPRIPOX VIRUS NATIONAL REFERENCE LABORATORIES WORKSHOP 2017

**EU Reference Laboratory
for Peste des Petits Ruminants**



**EU Reference Laboratory
for Capripox viruses**



5-6 October 2017

At CODA-CERVA

Belgium



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LSD Vaccines



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In vivo evaluation of Lumpy Skin Disease vaccine efficacy in controlled environment

BILL & MELINDA
GATES foundation



Protecting Livestock – Improving Human Lives

service public fédéral
SANTÉ PUBLIQUE,
SECURITE DE LA CHAÎNE ALIMENTAIRE
ET ENVIRONNEMENT



federale overheidsdienst
VOLKSGEZONDHEID,
VEILIGHEID VAN DE VOEDSELKETEN
EN LEEFMILIEU



European
Commission

THE
Pirbright
INSTITUTE



LIÈGE
université

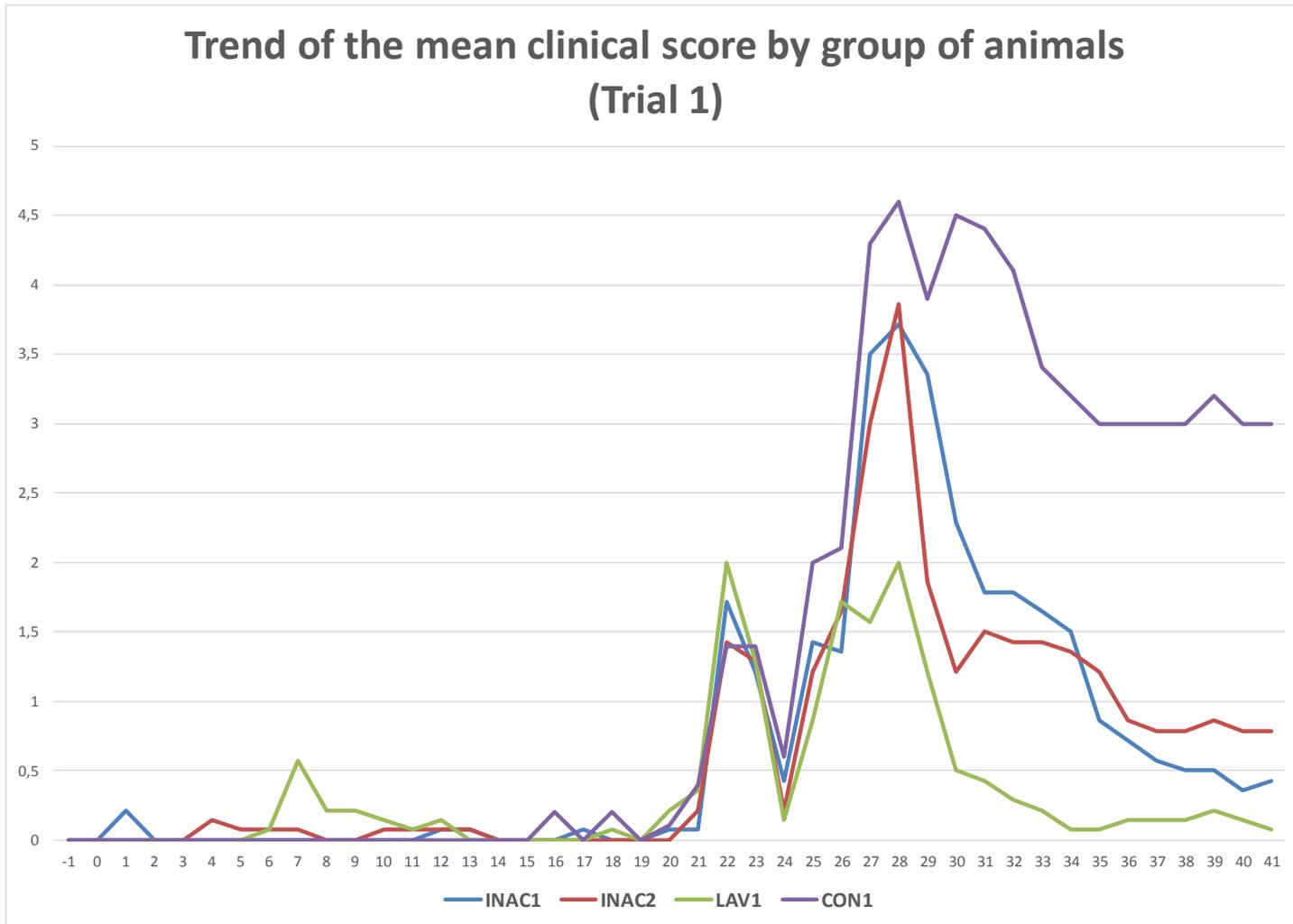
Vaccine Trial

INACTIVATED LSD Vaccines

1-2-3



Trend of the mean clinical score by group of animals
(Trial 1)



Control group

INAC 1

INAC 2

INAC 3

(New) Available Vaccines

- **New Inactivated LSD Vaccine (MCI, Morocco)**
 - LSDV-based
 - **Full Protection after Booster vaccination** (experimental trial)
 - Field trials in progress: Bulgaria?
- **Commercial available → Live attenuated vaccines (LAV)**
 - LSDV-based
 - ✓ OBP (Onderste Poort; South-Africa)
 - ✓ LumpyVax (MSD; South-Africa)
 - ✓ **Bovivax (MCI, Morocco + Huvepharma, Hungary)**
 - Important to know site effects in primo-vaccinated animals in the field (Serbia, Bulgaria)?

WARNING: Lumpyvax < > Lumpivax
MSD Kenya (KEVEVAPI)



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Production of Reference Material and LSD Studies

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Studies

- Production of Reference Material
 - Serology: positive control
 - Virology
 - Positive control virus isolation
 - Positive control Molecular tests
 - Positive control DIVA test (Neethling disease)
- Biological Material (serology/virology)
 - Portugal - the Netherlands
 - Spain - Italy
 - Denmark - France
 - Germany

Studies

- Duration of Immunity and of Protection
- Transmission studies
 - Vectors
 - Direct transmission
- Vaccine Quality control
 - Laboratory tests
 - EMA
 - Meeting on availability of LSD vaccines authorised to EU standards, 31 January 2017
 - Meeting on field efficacy trials for veterinary vaccines, 22-23 June 2017
 - OMCL / EDQM



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH



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Vaccine Quality Control



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.be

Quality Control Vaccines

- Role of Competent Authorities (CA):

Federal Agencies for Medicines

Ensuring that the marketed products comply with the specification laid down in the approved application file.

- Role of an OMCL:



= **Official Medicines Control Laboratory**
for **Immunological Veterinary Medicinal Products**

Support the CA in controlling the quality of medicinal products on the market

– Independent from manufacturers

– **Veterinary Batch Release Network (VBRN)**

<https://www.edqm.eu/en/ocabrobpr-immunological-veterinary-medicinal-products-ivmps>

VBRN Activities related to LSDV vaccines

1. **Awareness** of situation LSDV vaccines amongst Members + request to **inform** National Competent Authorities involved
2. **Identification** of potential VBRN contributions to ensure LSDV vaccines can be used in a safe and efficacious way despite lack of legal framework related to Official Control Authority for Batch Release
e.g. protocol review (if available) + performance relevant testing
3. **Creation of a list of relevant tests** for QC, based upon formal guidelines and risk evaluation of present vaccines
neither exhaustive, nor formal recommendation
-> each MS considers which tests to be important on vaccines entering their territory

VBRN Activities related to LSDV vaccines

4. **Laboratory Competency/Capacity** determined within VBRN related to testing list

5. **Sharing** of list relevant tests + lab capacities in VBRN with:
 - VBRN members
 - Head of Medicines Agencies (CA)
 - Upon request: non-EU countries, provided suitable confidentiality agreements are in place

- Contact list VBRN:

https://www.edqm.eu/sites/default/files/annex_iv_contact_list_ivmps.pdf

Veterinary Batch Release Network (VBRN)

= Representatives of:

- European Union Member States (MS) (*)
- European Economic Area (EEA) Member States (*)
- Mutual Recognised Partners (*)
- European Commission: DG Health and Food Safety
- European Medicines Agency (EMA)
- European Directorate for the Quality of Medicines (EDQM) ⌘

<https://www.edqm.eu/en/ocabrobpr-immunological-veterinary-medicinal-products-ivmps>

(*) Competent Authorities, including OMCL for IVMPs

⌘ Secretariat to the VBRN





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Thank you for your attention!

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VBRN Activities related to LSDV vaccines

4. Competency/capacity determined within VBRN related to testing list

Country		Institute	
BE	Belgium	CODA-CERVA	Veterinary and Agrochemical Research Centre
CH	Switzerland	IVI	Institut für Viruskrankheiten and Immunoprophylaxe
GE	Germany	PEI	Paul-Ehrlich-Institut
HU	Hungary	NFCSSO	Directorate of Veterinary Medicinal Products

Information about VBRN

- Activities:

<https://www.edqm.eu/en/ocabrobpr-immunological-veterinary-medicinal-products-ivmps>

- Contact list VBRN:

https://www.edqm.eu/sites/default/files/annex_iv_contact_list_ivmps.pdf



ANNEX IV

CONTACT LIST FOR THE NETWORK OF COMPETENT AUTHORITIES FOR
IVMPS