

DIAGNOSTICS LUMPY SKIN DISEASE

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Lumpy skin disease: Characteristic clinical signs

- Fever
- Skin nodules
- Drop in milk production





Laboratory confirmation:

- "Gold standard" serological test: serum/virus neutralization test (SNT/VNT).
 Not all animals either naturally infected or vaccinated develop LSDV neutralizing antibodies.
- Enzyme-linked immunosorbent assay (ELISA) by IDVet (France).
- Conventional and Real-Time Polymerase chain reaction (PCR) assays.
- Virus isolation on cell culture (Skin nodules).



Lumpy skin disease: WOAH Manual: B. DIAGNOSTIC TECHNIQUES

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Vov	Method	Purpose						
Key: +++ = recommended for this purpose		Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination	
	Detection of the agent							
++ recommended but has limitations	Virus isolation	+	++	+	+++	+	_	
	PCR	++	+++	++	+++	+	_	
+ = suitable in very limited circumstances	Transmission electron microscopy	_	—	—	+	_	_	
	Detection of immune response							
 – = not appropriate for this purpose 	VNT	++	++	++	++	++	++	
	IFAT	+	+	+	+	+	+	
	ELISA	++	++	++	++	++	++	

PCR = polymerase chain reaction; VNT = virus neutralisation test;

IFAT - indirect fluereceast antibady test. FLICA - ensure linked instrume carbont econy

Lumpy skin disease: Molecular test to differentiate vaccine and wild-type LSDV

DIVA: Differentiation of Infected from Vaccinated Animals

Differentiate between Cluster 1.1 and 1.2 Quantitative real-time PCR assays have been designed to differentiate between Neethling-based LSDV strains, which are often used for vaccination, and wild-type LSDV strains from cluster 1.2 (Agianniotaki et al., 2017; Pestova et al., 2018; Vidanovic et al., 2016).

These DIVA assays (enable, for example, differentiation of "Neethling response" caused by vaccination with a LSDV Neethling vaccine strain from disease caused by infection with a cluster 1.2 wildtype virus.

		KX764644 LSDV Herbivac vaccine	7		
		MG972412 LSDV Cro2016 Croatia 2016			
		KX764643 LSDV SIS-Lumpyvax vaccine			
		AF409138 LSDV Neethling vaccine LW 1959			
		KX764645 LSDV OBP vaccine			
	10	MK441838 LSDV Herbivac LS-batch-008 vaccine			
		OM793609 LSDV Vaccine LW-1959 1988			
		MW656252 LSDV Haden RSA 1954			
		OM793608 LSDV Neethling-WC RSA 1957			
		OM793606 LSDV Potter RSA 1958	LSDV Cluster 1.1		
	100	OM793607 LSDV Fourie-FS RSA 1959			
		OM793605 LSDV Hoffmeyer RSA 1958			
		OM793604 LSDV 33-KZN RSA 1977			
		MN636839 LSDV 103-GP RSA 1991			
	100	MN636843 LSDV 148-GP RSA 1997			
	100	MN636838 LSDV 58-LP RSA 1993			
	10	MN636840 LSDV 248-NW RSA 1993			
	100	MN636841 LSDV 220-1-NW RSA 1993			
		MN636842 LSDV 220-2-NW RSA 1993			
	96	MH646674 LSDV Saratov Russia 2017	LSDV Recombinant		
	100 MV	/355944 GD01 China 2020	LSDV Recombinant		
	МТ992	2618 LSDV Kostanay Kazakhstan 2018	LSDV Recombinant		
	MT13404	2 LSDV Udmurtya Russia 2018	LSDV Recombinant		
100	00 KX683219 LS	DV KSGPO-240 Kenya 1959	7		
	AF325528 LSDV NI-2490 Kenya 1958				
	AF409137 LS	SDV Warmbaths-LW RSA 2000			
1	100 MW656253 LSDV 280-KZN RSA 2018				
	MH893760 LSDV Neethling Russia 2015				
	KY829023 LSDV Evros Greece 2015				
	MT643825 LSDV 210-249 Bulgaria 2016 LSDV Cluster 1.2				
	KY702007 LS	SDV Bujanovac Serbia 2016			
	¹⁰⁰ MN995838 LSDV Pendik Turkey 2014				
	KX894508 LSDV Neethling 155920 Israel 2012				
	MN642592 LSDV Kubash Kazakhstan 2016				
	MT130502 LS	SDV Neethling-RIBSP vaccine			
	100	Goatpox virus			
		─ Sheeppox virus			

Lumpy skin disease: Molecular test to differentiate vaccine and wild-type LSDV

DIVA: Differentiation of Infected from Vaccinated Animals

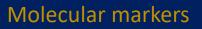
CAN NOT

differentiate between vaccine and wild type isolates within Cluster 1.1

CAN NOT differentiate between vaccines and novel recombinant strains (Cluster 2.1 – 2.5) However, these DIVA PCR assays cannot distinguish between a LSDV Neethling vaccine strain and the novel recombinant LSDV strains recently isolated from disease outbreaks in Asia (Byadovskaya et al., 2021; Flannery et al., 2021). These DIVA assays are also not capable of discriminating between LSDV Neethling vaccine strains and recently characterised (historic) wild-type viruses from South Africa belonging within cluster 1.1 (Van Schalkwyk et al., 2020; 2021).

				KX764644 LSDV Herbivac vaccine	-	
				MG972412 LSDV Cro2016 Croatia 2016		
				KX764643 LSDV SIS-Lumpyvax vaccine		
				AF409138 LSDV Neethling vaccine LW 1959		
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				MN636842 LSDV 220-2-NW RSA 1993	_	
	98		- N	1H646674 LSDV Saratov Russia 2017	1 -	LSDV Recombinant
	100		MW	355944 GD01 China 2020	=	LSDV Recombinant
		– M	T992	618 LSDV Kostanay Kazakhstan 2018	=	LSDV Recombinant
	MT134042 LSDV Udmurtya Russia 2018 ISDV Recombinant					LSDV Recombinant
100	₀₀ KX683	3219	9 LSI	DV KSGPO-240 Kenya 1959	-	
	AF325528 LSDV NI-2490 Kenya 1958					
	AF40	913	7 LS	DV Warmbaths-LW RSA 2000	1	
1	100 MW656253 LSDV 280-KZN RSA 2018					
	MH893760 LSDV Neethling Russia 2015					
	L KY829023 LSDV Evros Greece 2015 LSDV Cluster 1.2					
	MT643825 LSDV 210-249 Bulgaria 2016					
	KY702007 LSDV Bujanovac Serbia 2016					
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	MN642592 LSDV Kubash Kazakhstan 2016					
MT130502 LSDV Neethling-RIBSP vaccine						
	100			Goatpox virus		
				◄ Sheeppox virus		

Lumpy skin disease: Epidemiology



GPCR: TaqMan; (Agianniotaki et al., 2017)

RPO30

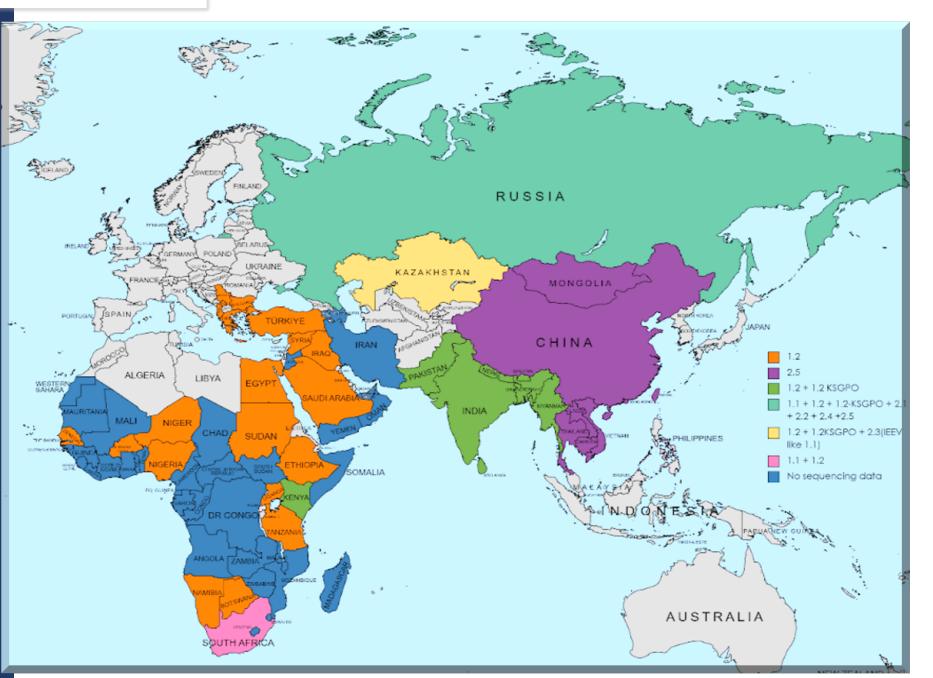
LW008: TaqMan; (Vidanovic et al., 2021)

EEV (Agianniotaki et al., 2021)

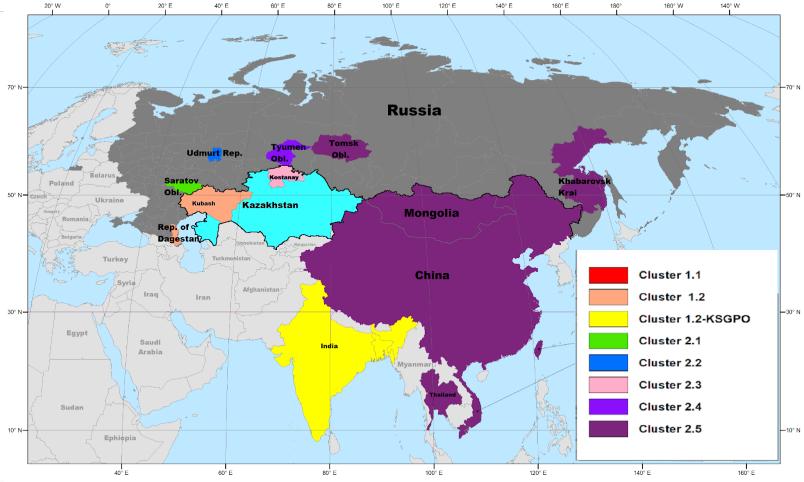
P32

LW044: TaqMan (Sprygin et al., 2019)

Complete genomes



Consequently, in regions where recombinant strains (currently Asia and possibly elsewhere) or wildtype cluster 1.1 strains are circulating (currently South Africa and possibly elsewhere) are circulating, these DIVA assays are not suitable for distinguishing vaccine and wild-type virus. Thus, in order to overcome these constraints, whole genome sequencing is recommended.





Kumar et al., 2023

HRM-based gap-qRT-PCR: 801bp in terminal repeat region (ITR)

Vaccine: (Lumpi-ProVac^{Ind}) vs. Wild type: (LSDV/2019/India/Ranchi)

Haegeman et al., 2023

Duplex qRT-PCR: LW133 and LW144

• Vaccine (Neethling) vs. Wild type: Cluster 1.2 vs. Recombinant (Cluster 2.5)

Krotova et al., 2023

PCR and Sanger sequencing: 705bp in ORF LW134

 Vaccines (Neethling and KSGPO) vs. Wild type Cluster 1.2 vs. Recombinant (Cluster 2.1, 2.2, 2.3, 2.4 and 2.5)



Nan et al., 2023	Triplex real-time PCR:LSDV vs GTPV vs SPPV
Liao et al., 2023	CRISPR-Cas12a: • LSDV vs GTPV vs SPPV
Nandi et al., 2023	Isothermal PCR: 27bp in ORF LW126 • Vaccines (GTPV) vs. Wild type Cluster 1.2-KSGPO
Abdalhamed et al., 2022	Gold nanoparticle – lateral flow test
Sthitmatee et al., 2023	In-house ELISA using whole virus (LSD/THA/CMU/21/05)



