ASF EURL presentation of interlaboratory coordination and support on ASF diagnosis to Western Balkan countries

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Twenty-third Meeting of the Standing Group of Experts on African swine fever in Europe (SGE ASF23)
Transboundary cooperation in African swine fever control
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Centro de investigación en Sanidad Animal (CISA/INIA-CSIC). Madrid, Spain

DUTIES of the ASF-EURL
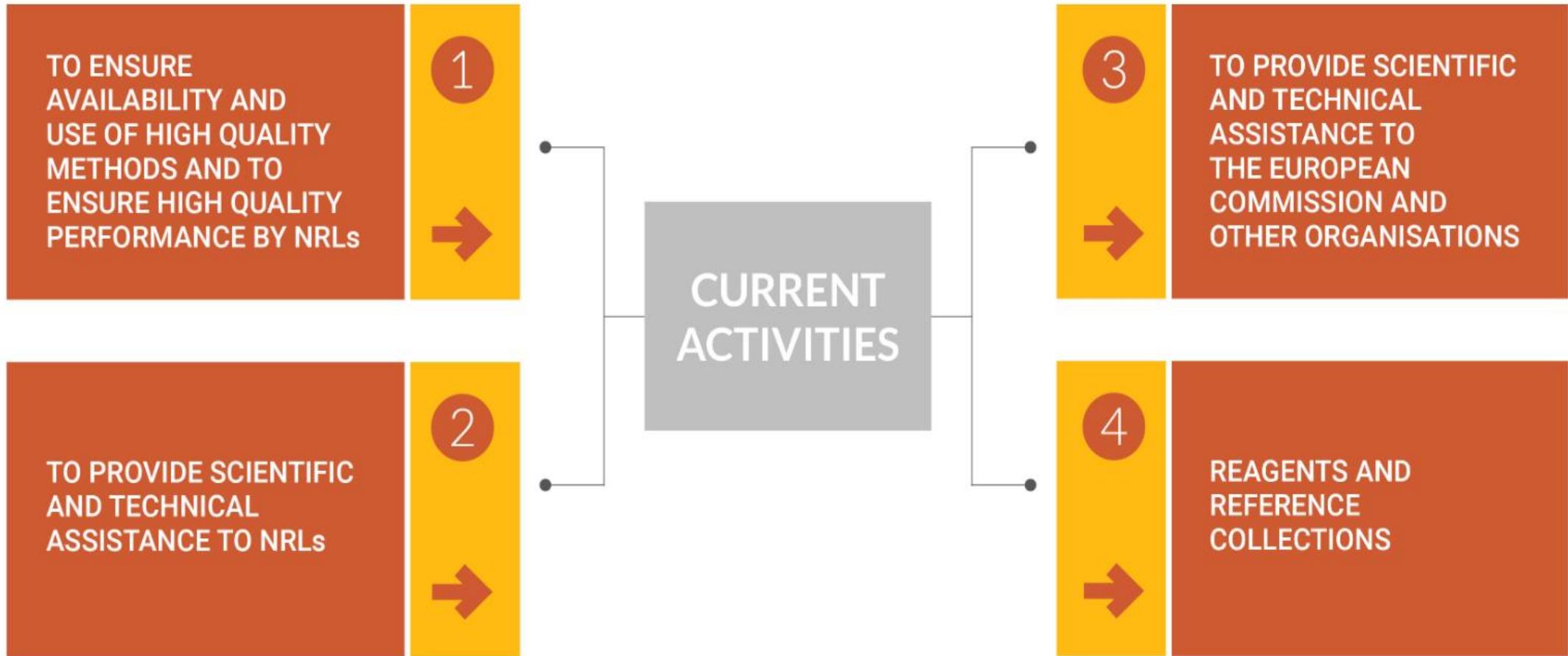
EUROPEAN UNION REFERENCE LABORATORY FOR
AFRICAN SWINE FEVER

1 TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

CURRENT ACTIVITIES

3 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS

4 REAGENTS AND REFERENCE COLLECTIONS

DUTIES of the ASF-EURL

1 **TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs**

⬇

Organization of the **annual Inter-Laboratory comparison test (ILCT)** for ASF.

⬇

OBJECTIVE: to harmonise and optimise the serological and virological techniques for ASF used in the national reference laboratories (NRLs) in order to ensure that laboratory tests to detect the presence of ASF are carried out in each Member State and other official laboratories in accordance with EC Directives and Regulations.

ILCT: What does it consist of?

1 **Selection of a representative sample panel of all epidemiological situations.**

| ID SAMPLE	CLINICAL FORM	VIRULENCE ASFV	ASFV ISOLATE	GENOTYPE	ORIGIN OF SAMPLES	
					DPI (days post infection)	DESCRIPTION
S1				Naive pig (ASF negative)		
S2	ACUTE	VIRULENT	LT14/1492	II	D18	Pig kept in contact with pigs i.m. inoculated with 10 HAU/ml Lithuanian ASFV isolate LT14/1492. Serum obtained at 18 dpi.
S3	ACUTE	VIRULENT	Ken06.Bus	IX	D12	Dilution 1/8 in negative serum of 1x Kenyan ASFV isolate Ken06.Bus i.m (10 HAU/ml) Serum obtained at 12dpi.
S4	CHRONIC	ATTENUATED	NH/P68	I	D97	1x Portuguese ASFV isolate NH/P68 i.m (103 TCDI50/ml) Serum obtained at 97 dpi.
S5	CHRONIC	ATTENUATED	NH/P68	I	D42	Pig keep in contact with pigs inoculated with 1x Portuguese ASFV isolate NH/P68 i.m. (105 TCDI50/ml). Serum obtained at 42 dpi.
S6	ACUTE	VIRULENT	Arm07	II	D8	1x Armenian ASFV isolate Arm07 i.m. (10 HAU/ml). Serum obtained at D8 dpi.
S7				Naive pig (ASF negative)		
S8	SUBACUTE	MODERATE	Ken05/Tk1	X	D70	1x Kenyan ASFV isolate Ken05/Tk1 i.m. (10 HAU/ml). Serum obtained at 70 dpi.
S9	ACUTE	VIRULENT	Benin/97	I	D7	1x Benin ASFV isolate i.m. Benin/97 (10 HAU/ml). Serum obtained at 7 dpi.
S10	CHRONIC	ATTENUATED	NH/P68	I	D105	1x Portuguese ASFV isolate NH/P68 i.m. (105 TCDI50/ml). Serum obtained at 105 dpi.
S11	ACUTE	VIRULENT	LT14/1492	II	D17	Pig kept in contact with pigs i.m. inoculated with 10 HAU/ml Lithuanian ASFV isolate LT14/1492. Serum obtained at 17dpi.
S12	CHRONIC	ATTENUATED	NH/P68	I	D41	1x Portuguese ASFV isolate NH/P68 i.m. (105 TCDI50/ml). Serum obtained at 41 dpi.
S13	CHRONIC	ATTENUATED	NH/P68	I	D105	1x Portuguese ASFV isolate NH/P68 i.m. (105 TCDI50/ml). Serum obtained at 105 dpi.
S14				Naive pig (ASF negative)		

The panel includes around 20 sera and tissues collected from pigs infected with ASFVs of different genotypes and virulence that induce various clinical forms.

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ILCT: What does it consist of?

2 Sample delivery→ **36 participants in 2023-2024**

26 NRLs from EU Member States
8 NRLs from Europe non-EU Member states
1 non NRL from EU Member State
1 NRL from non-European country

ALL EU NRLs from Member States PARTICIPATE in the ILCT *(1NRL have ASF diagnosis transferred to neighbouring NRLs belonging to MS country)*
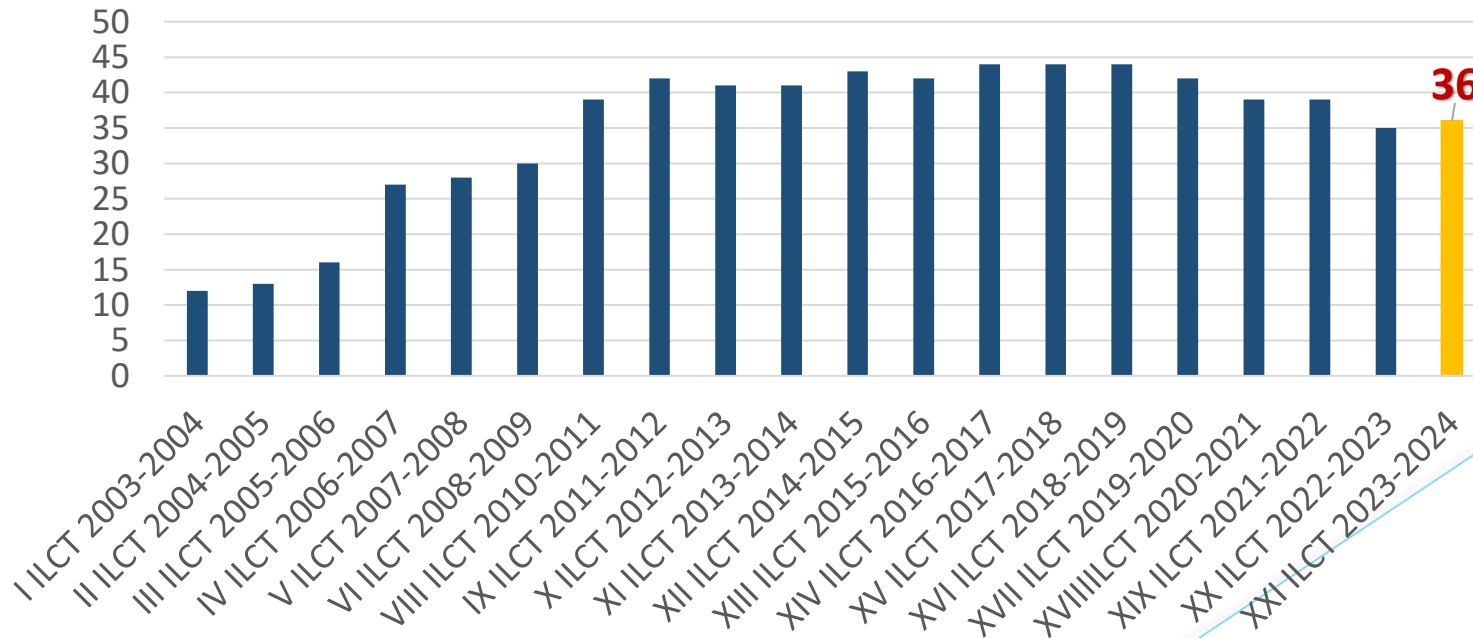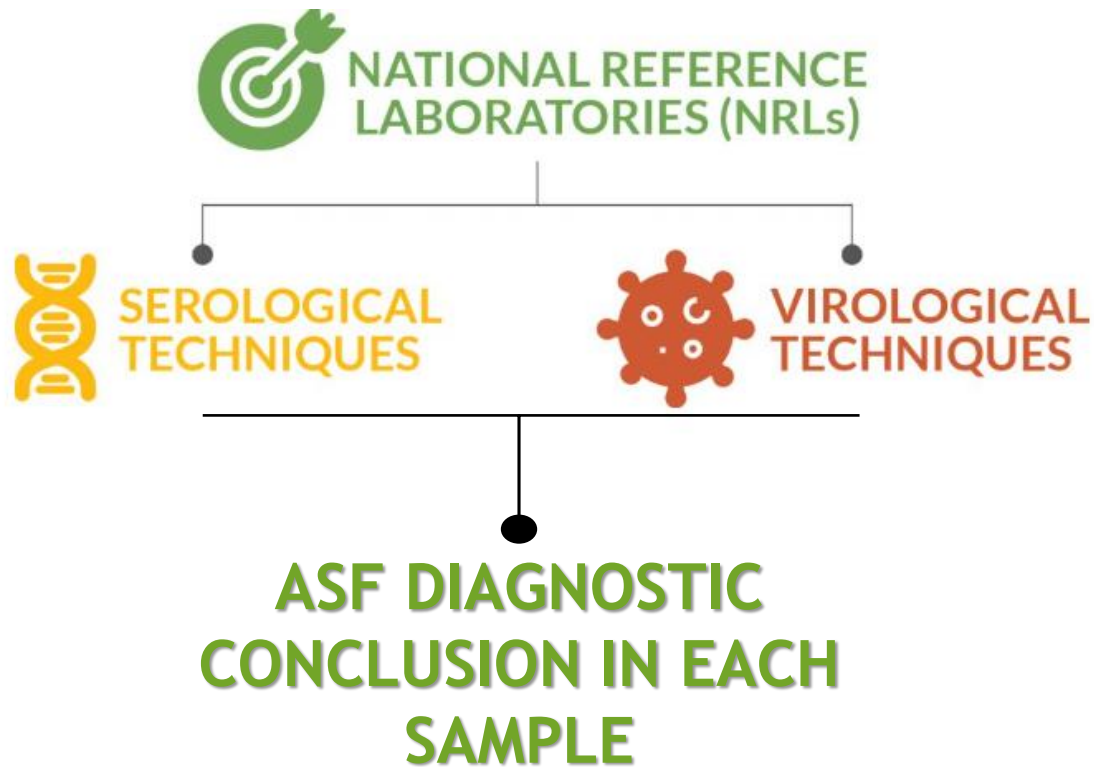
DUTIES of the ASF-EURL

ILCT: What does it consist of?

2 **Sample delivery→ Western Balkan countries**

ILCT	ALBANIA	Bosnia and Herzegovina	Kosovo	NORTH MACEDONIA	MONTENEGRO	SERBIA
XI-ILCT 2013-2014	Z	Z	Z	Z	Z	Yes
XII-ILCT 2014-2015	Z	Z	Z	Z	Z	Yes
XIII-ILCT 2015-2016	Z	Z	Z	Z	Z	Yes
XIV-ILCT 2016-2017	Z	Z	Z	Z	Z	Yes
XV-ILCT 2017-2018	Z	Z	Z	Yes	Z	Yes
XVI-ILCT 2018-2019	Z	Z	Z	Yes	Z	Yes
XVII-ILCT 2019-2020	(-)	(-)	(-)	Yes	Yes	Yes
XVIII-ILCT 2020-2021	(-)	Yes	Yes	Yes	Yes	Yes
XIX-ILCT 2021-2022	(-)	Yes	Yes	Yes	Yes	Yes
XX-ILCT 2022-2023	(-)	(-)	Yes	Yes	Yes	Yes
XXI-ILCT 2023-2024	(-)	Yes	Yes	Yes	Yes	Yes

- Yes→ the NRL received the panel of samples and sent the results on time
- X→ the NRL received the panel of samples but didn't send the results on time
- (-) → the NRL didn't participate.
- (Z) → Not invited

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ILCT: What does it consist of?

③ **Analysis of the results**

NATIONAL REFERENCE LABORATORIES (NRLs)

SEROLOGICAL TECHNIQUES

VIROLOGICAL TECHNIQUES

ASF DIAGNOSTIC CONCLUSION IN EACH SAMPLE

XXI-Interlaboratory trial of ASF serology and virology - Results template				
COUNTRY: ID CODE: LABORATORY: DATE ANALYSIS: CONTACT PERSON: email address:				
		Ab detection	Virus detection	ASF diagnostic conclusion
SERA	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			
	11			
	12			
	13			
	14			
	15			
Tissues	1			
	2			
	3			
	4			
	5			

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ILCT: What does it consist of?

3 Analysis of the results→ **Type of techniques employed**

Serological techniques (all participants)

❖ **ALL labs** performed the ASF serological diagnosis.

❖ **Techniques:**
  ✓ **Screening: INGENASA ELISA K3** is the technique most widely used for ASF antibody detection **(89%).**
  ✓ **Confirmatory test: 75%** of participants have used at least one confimatory test.
    • The **immunoperoxidase technique (IPT)** is the chosen procedure **(81.5%).**
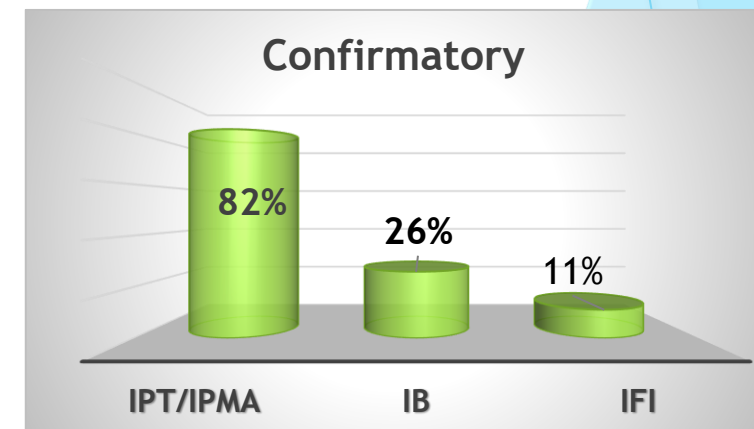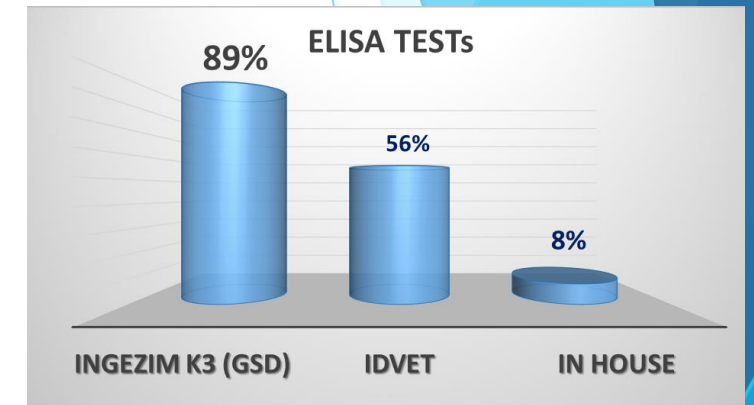
ELISA TESTs

89% · 56% · 8%

INGEZIM K3 (GSD) · IDVET · IN HOUSE

Confirmatory

82% · 26% · 11%

IPT/IPMA · IB · IFI

DUTIES of the ASF-EURL

ILCT: What does it consist of?

3 Analysis of the results→ **Type of techniques employed**

Serological techniques (Western Balkan countries)

XXI-ILCT 2023-2024	Bosnia and Herzegovina	Kosovo	NORTH MACEDONIA	MONTENEGRO	SERBIA
ELISA	Ingezim PPA-K3	IDVET	IDVET	Ingezim PPA-K3	Ingezim PPA-K3
CONFIRMATORY TEST	NO	**NO**	**NO**	Yes	**IB**

All labs have at least the ELISA test for ASF antibody detection as a minimum requirement.

ILCT: What does it consist of?

3 Analysis of the results→ **Type of techniques employed**

Virological techniques (all participants)

- PCR
- VI
- ELISA-AG
- GENOTYPING

100% 19% 17% 22%
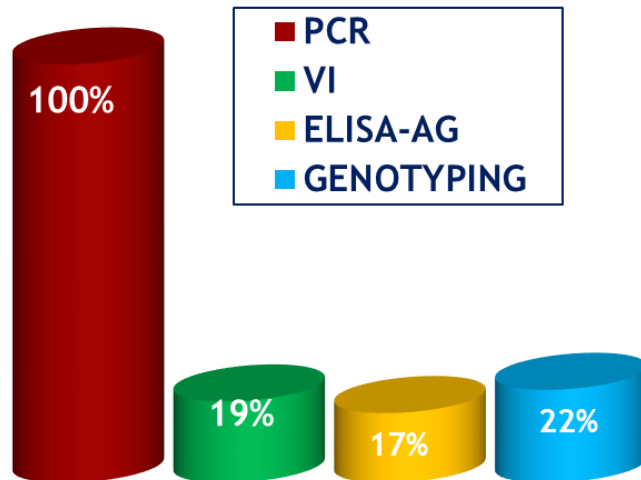
❖ **ALL participating laboratories** performed the virological diagnosis in serum and tissue samples.

❖ **Techniques: PCR test** ALL participants use it for ASFV detection **(100%).**

  • **The 58%** of participants have used more than one PCR test.

  • **All labs** used at **least one Real-Time PCR** while **conventional PCR** was performed by **19%**

  • The **UPL real time PCR** is the **most widely used (56%).**

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EUROPEAN UNION REFERENCE LABORATORY FOR **AFRICAN SWINE FEVER**

ILCT: What does it consist of?

3 Analysis of the results→ **Type of techniques employed**

Virological techniques (Western Balkan countries)

XXI-ILCT 2023-2024		MONTENEGRO	KOSOVO	BOSNIA	SERBIA	NORTH MACEDONIA
Conventional PCR		(-)	(-)	(-)	(-)	Yes
Real time PCR	WOAH PCR [a]	**Yes**	**Yes**	(-)	(-)	Yes
	WOAH UPL PCR [b]	(-)	(-)	(-)	Yes	Yes
	In house	(-)	(-)	(-)	(-)	Yes
	Commercial	(-)	(-)	Yes	(-)	(-)
Ag-ELISA		(-)	(-)	Yes	(-)	(-)

[a] **WOAH- real time PCR**→ real time PCR (King et al., 2003) technique as is described in the WOAH Manual of diagnosis for ASF (Chapter 3.9.1. WOAH edition 2021).

[b] **WOAH-UPL-PCR**→ universal probe library (UPL) real time PCR developed by Fernández et al., 2013 described in the WOAH Manual of diagnosis for ASF (Chapter 3.9.1. WOAH edition 2021).

All labs have the PCR test for ASFV genome detection as a minimum requirement.

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AFRICAN SWINE FEVER

ILCT: What does it consist of?

3 Analysis of the results→ **Performance of techniques employed**

SEROLOGICAL
TECHNIQUES

VIROLOGICAL
TECHNIQUES

ASF DIAGNOSTIC
CONCLUSION IN EACH
SAMPLE

✓ ➢ **ELISA test** → The results obtained by all labs were correctly scored

✓ ➢ **Confirmatory tests** → The results obtained by all labs were correctly scored

✓ ➢ **Antibody detection conclusion** → correctly scored by ALL labs in positive and negative samples.

EUROPEAN UNION REFERENCE LABORATORY FOR
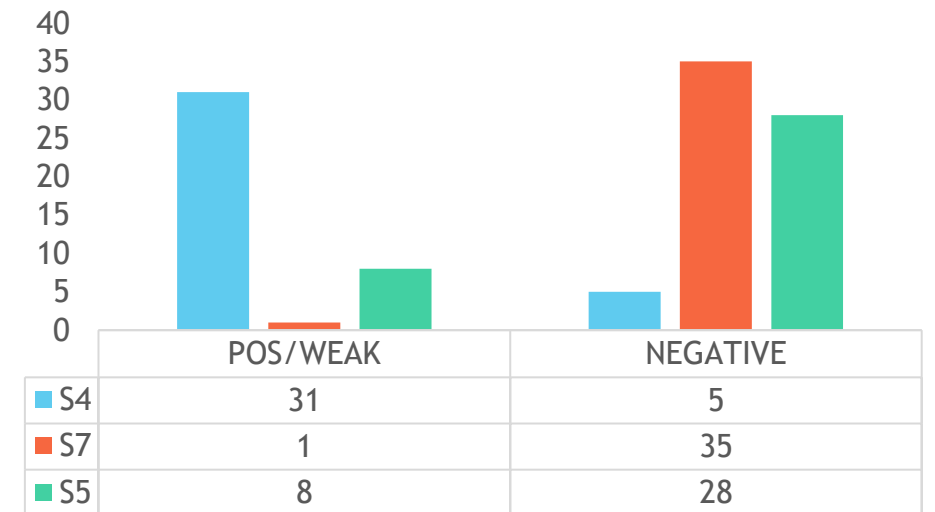**AFRICAN SWINE FEVER**

ILCT: What does it consist of?

3 Analysis of the results→ **Performance of techniques employed**

SEROLOGICAL TECHNIQUES

DEVIATIONS IN WEAK Ab POSITIVE SAMPLE
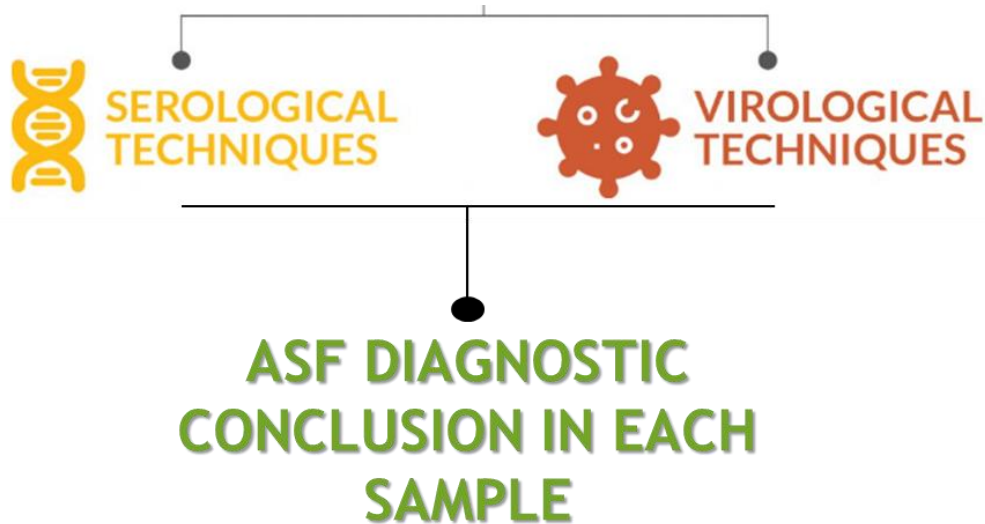**(low Ab titer)**

➢ **S1** → The results were correctly scored by **the 47,2% of participant labs (17/36).**

➢ **S2** → The results were correctly scored by **the 11,1% of participant lab (4/36).**

➢ **S3** → The results were correctly scored by **the 47,2% of participant labs (17/36).**

	S1	S8	S13
POSITIVE/WEAK	17	4	17
NEGATIVE	19	32	19
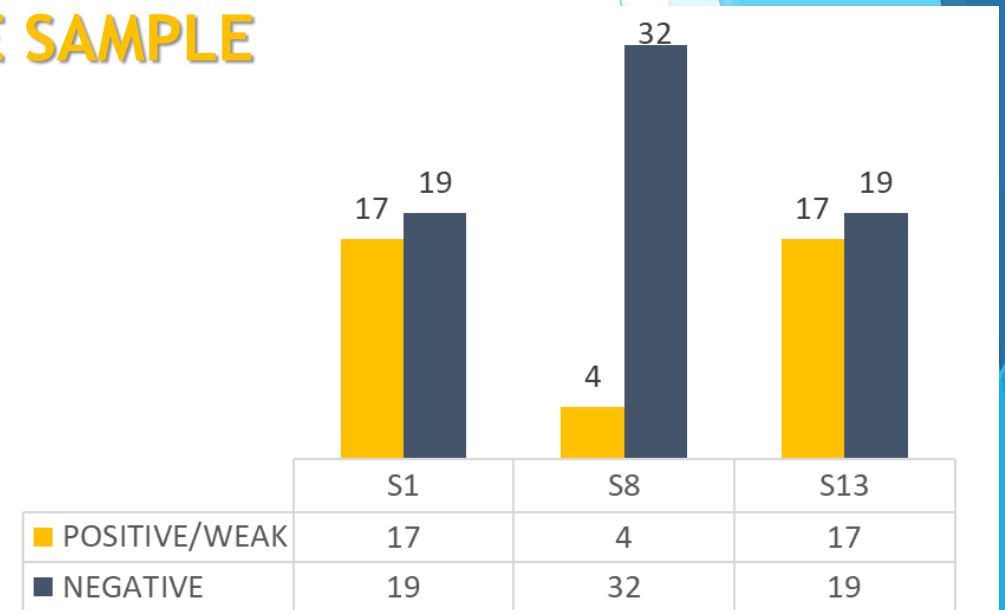
These samples are strong PCR positive samples

ILCT: What does it consist of?

3 Analysis of the results→ **Performance of techniques employed**

SEROLOGICAL TECHNIQUES

VIROLOGICAL TECHNIQUES

ASF DIAGNOSTIC CONCLUSION IN EACH SAMPLE
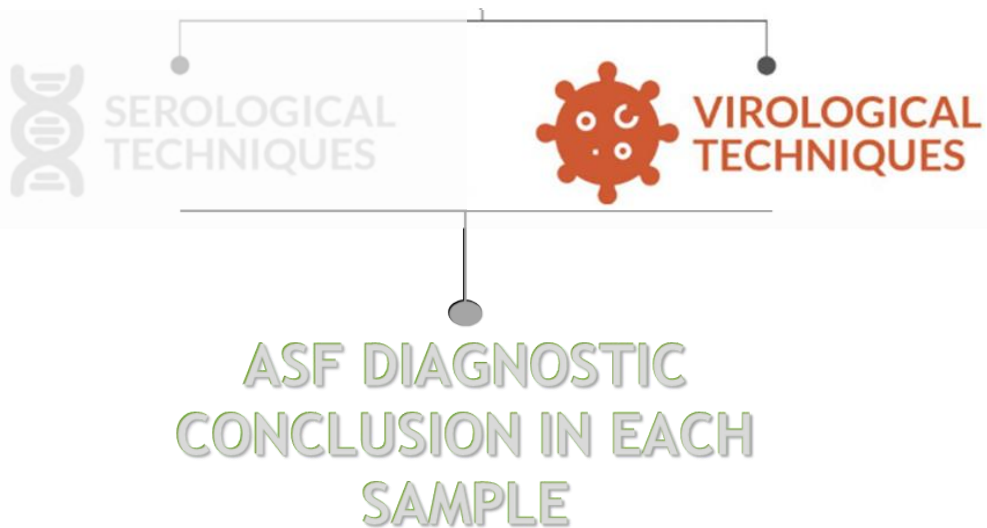
✓ ➤ **Virus detection conclusión (PCR test) →** correctly scored by ALL labs in positive and negative samples.

ILCT: What does it consist of?

3 Analysis of the results→ **Performance of techniques employed**

VIROLOGICAL TECHNIQUES

DEVIATIONS IN WEAK PCR POSITIVE SAMPLE
(low ASFV genome content)

➢ **S4** → The results were correctly scored by **the 86,1% of** participant labs (31/36).

➢ **S7** → The results were correctly scored by **the 2,7% of** participant lab (1/36).

➢ **S5** → The results were correctly scored by **the 22,2% of** participant labs (8/36).

	POS/WEAK	NEGATIVE
S4	31	5
S7	1	35
S5	8	28

These samples are strong Ab positive samples

EUROPEAN UNION REFERENCE LABORATORY FOR AFRICAN SWINE FEVER

From the XXI ASF ILCT 2024 results...

❖ The EURL concluded that the western Balkan countries that have participated in the ILCT, including **Serbia, North Macedonia, Bosnia, Kosovo, and Montenegro, are successfully applying diagnostic procedures that meet the necessary standards for accurate ASF diagnosis.**

❖ The NRLs **meet the minimum diagnostic requirements for ASF** by having at least **one antibody test (such as ELISA) and a PCR test in place**. These tools ensure effective monitoring and control of ASF in the region.

❖ Their methodologies have been deemed 'fit for purpose' by the EU Reference Laboratory for ASF.

Source: CSIS, NATO, EEAS, 2023.

DUTIES of the ASF-EURL

EUROPEAN UNION REFERENCE LABORATORY FOR AFRICAN SWINE FEVER

1 TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs

Supply of ASF reference standards, biological material and reagents for ASF diagnosis.

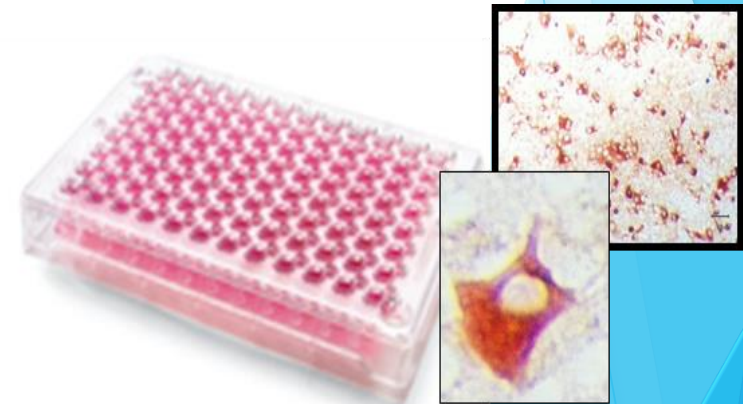| ASF REAGENTS	KOSOVO	BOSNIA	SERBIA
ASF-PC (ml)	1	2.1	2.1
ASF NC (ml)	1	2.1	2.1
ASF-LC (ml)	1	2.1	2.1
ASF-IPT plates/ analysis	-	3 /288	3 /288
HRP- Protein A (mg)	1	1	5
IB strips	100	53	202

DUTIES of the ASF-EURL

1 **TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs**

Supply of ASF-URL reference material for validation and internal verification of ASF diagnostic techniques.

- **Panel Ref-1: Ten URL-ASF reference serum samples** for the evaluation and internal verification of ASF **antibody detection techniques.**
- **Panel Ref-2: Sixteen URL-ASF reference samples** for the evaluation and internal verification of **DNA extraction methods.**
- **Panel Ref-3: Twenty one URL-ASF reference DNAs** for the evaluation and internal verification of **DNA amplification by PCR tests.**

	REF-1	REF-2	REF-3
BOSNIA	-	1	2
KOSOVO	-	1	1
MONTENEGRO	-	-	2
SERBIA	1	1	1
TOTAL	1	3	6

2

TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLS CONSISTING OF

Conducting **face to face and/or virtual training courses on ASF laboratory diagnosis** for staff from NRLs or other official labs.

Country	DATE	PARTICIPANTS	
		Nº	LABORATORY
MONTENEGRO	21-25 November 2016	1	Montenegro´s NRL
KOSOVO	25-29 November 2019	2	Kosovo Food and Veterinary Laboratory (Kosovo´s NRL)
SERBIA	15th to 19th April 2024	2	Serbia´s NRL

Three short term trainings on ASF diagnostic and Laboratory contingency plan (LCP) at the EURL INIA-CISA with the participation of staff from western Balkan countries

All NRLs are welcome to attend training courses at the EURL to enhance their diagnostic capacity if needed.

DUTIES of the ASF-EURL

3 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS THROUGH

Active assistance in the **diagnosis of the primary ASF outbreaks**

ASFV ISOLATE	COUNTRY	Collection Date	HOST
SER19/DP1	Serbia	30 July 2019	DP
BOS23/DP/1	Bosnia	22 June 2023	DP
MZ22/DP1	North Macedonia	10 January 2022	DP
MT24/WB1	Montenegro	12 January 2024	EWB

HAD VIRUSES

On going confirmatory diagnosis on samples received from Kosovo and Albania

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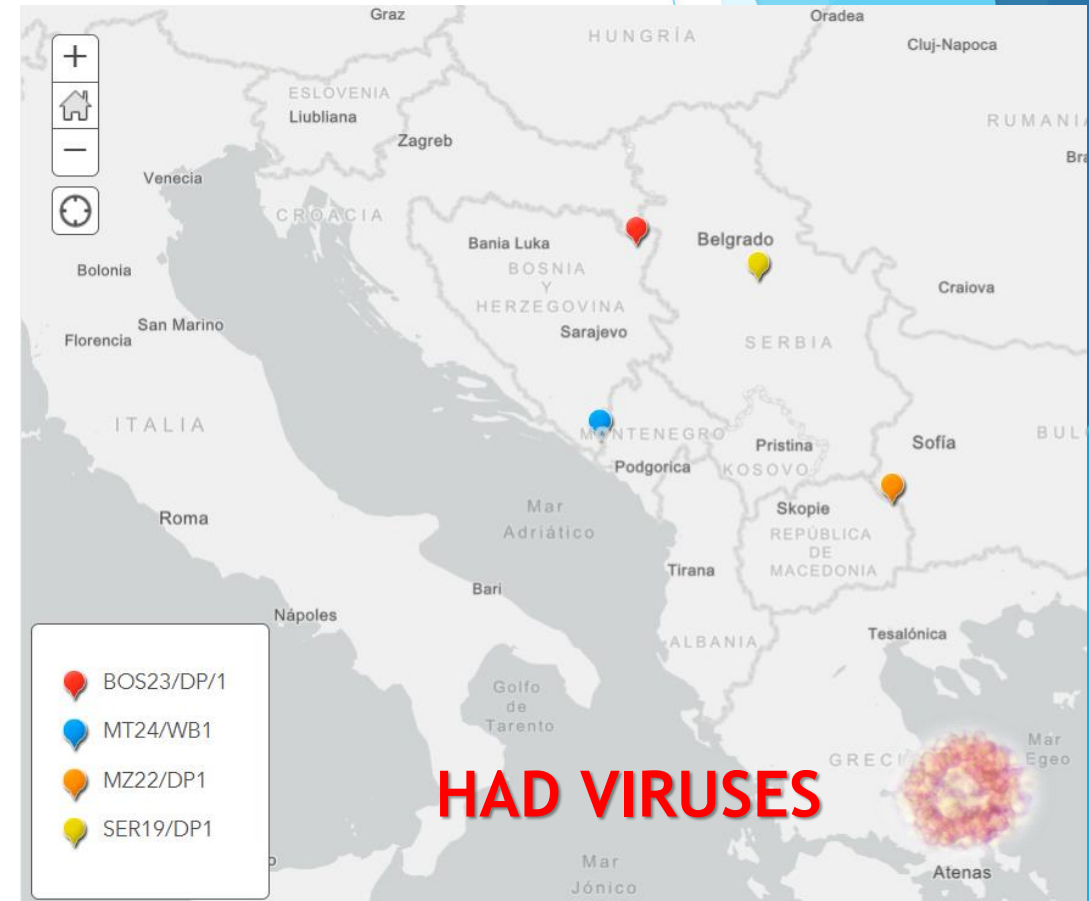
And **genetic characterization to trace the outbreaks**

The ASFVs from Western Balkan countries cluster in the **genotype II genetic group 19, the second largest one in the European countries**
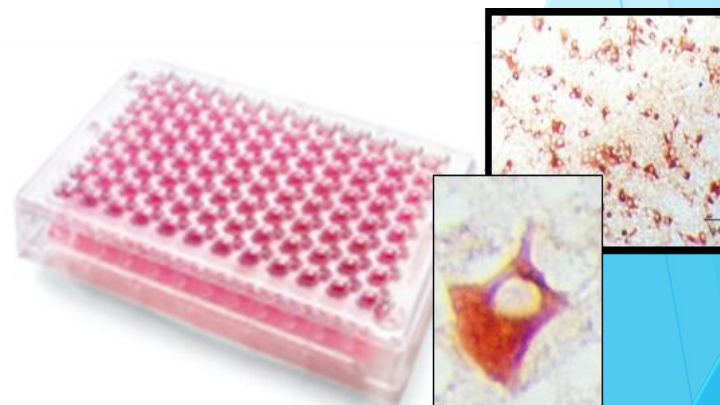

On going genetic characteristic on samples received from Kosovo and Albania

DUTIES of the ASF-EURL

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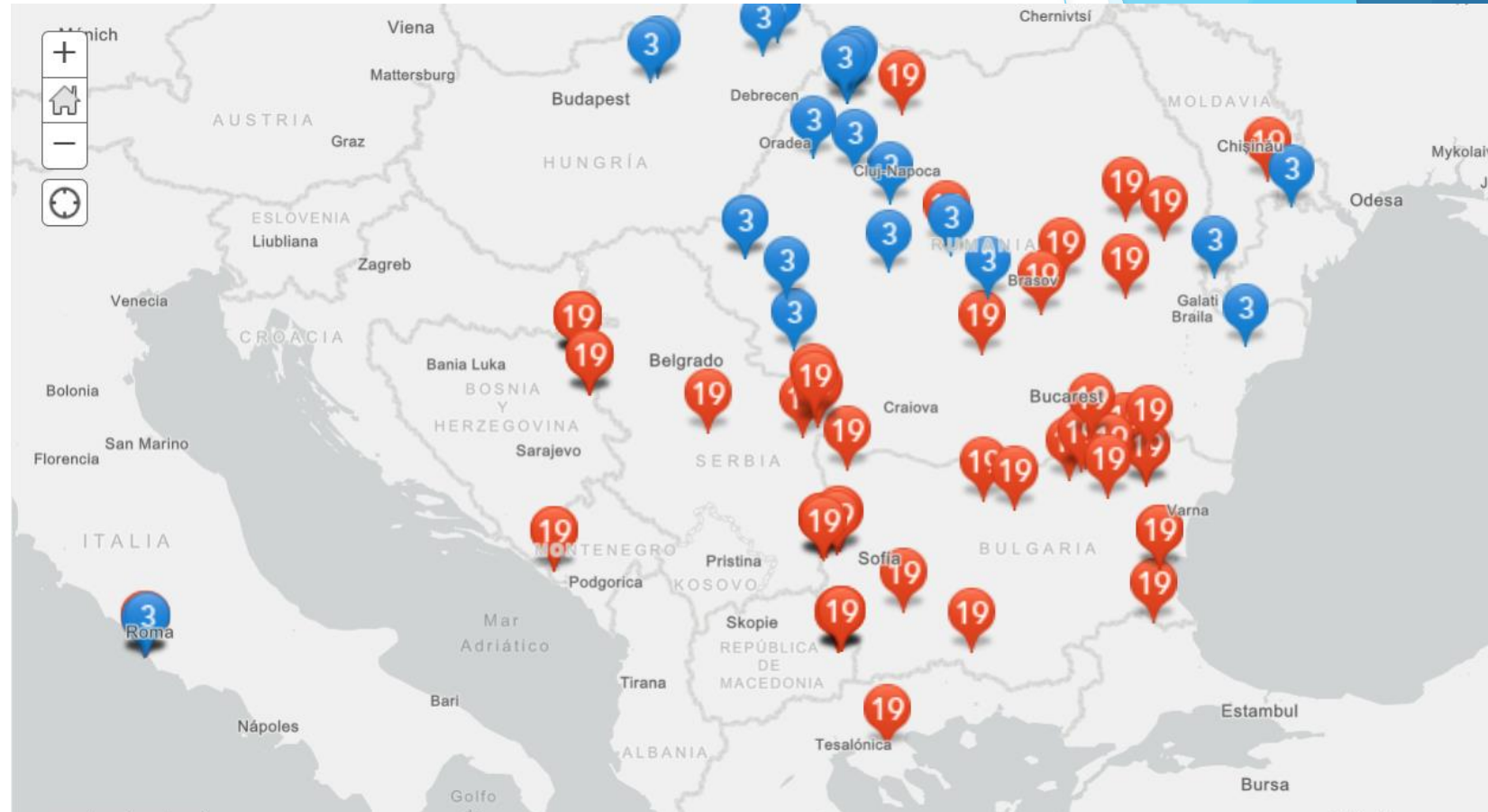
Genetic Group	Geographical distribution (year)	Genetic variants					
		CVR	IGR	O174L	K145R	MGF	ECO2
1	Georgia (2007), Armenia (2007,2008), Azerbaijan (2008), Russia Federation (2009,2012)	I	I	I	I	I	I
2	Russia Federation (2012).	I	I	I	I	II	I
3	Ukraine (2012-2019), Belarus (2013), Lithuania (2014-2022), Poland (2014, 2018, 2021), Latvia (2014-2021), Estonia (2014-2022), Czech RP (2017, 2018), Romania (2017-2021), Moldova (2017-2018), Hungary (2018-2019), Slovakia (2019), Italy (2022, 2023).	I	II	I	I	I	I
4	Russia Federation (2012).	I	I	I	I	III	I
5	Estonia (2015)	II	II	I	I	I	I
6	Poland (2016, 2019, 2021), Germany (2020, 2021) and Czech Republic (2022)	I	II	II	II	I	I
7	Poland (2016-2019, 2021), Lithuania (2017-2022), Romania (2019)	I	II	I	II	I	I
8	Poland (2016, 2017)	I	II	I	II	II	I
9	Estonia (2017)	I-SNP1	II	I	I	I	I
10	Poland (2017)	I	I	II	II	I	I
11	Poland (2017)	I-SNP2	II	I	II	I	I
12	Latvia (2017, 2018, 2021)	I	II	I	I	II	I
13	Poland (2017)	I	III	II	II	I	I
14	Lithuania (2017)	I-SNP3	II	I	I	I	I
15	Lithuania (2017)	I	II	I	I	V	I
16	Lithuania (2017, 2018)	I	II	I	I	IV	I
17	Latvia (2017, 2018)	I	II	I	I	I-V1	I
18	Poland (2018)	I	III	II	I	I	I
19	Romania (2018, 2021), Bulgaria (2018-2020), Serbia (2019, 2020), Greece (2020), North Macedonia (2022), Italy (2022), Croatia (2023, 2024), Sweden (2023), Montenegro (2024)	I	II	I	I	I	II
20	Poland (2018, 2019, 2021)	I	IV	I	II	I	I
21	Romania (2019)	I	II	II	I	I	I
22	Romania (2019)	I	II	II	I	I	II
23	Lithuania (2020)	I	II	I	I	VII	I
24	Romania (2021)	I	II	I	I	VI	I
25	Italia (Lazio-2023)	I	II	I	II	VIII	I
26	Italia (Piedmonte-2023)	I	II	I/SNP1	I	I	I
27	Poland (2021)	I	II	I	II	V	I
28	Estonia (2022)	I	II	I	I	IX	I

I215L mutation (ECO2 variant)

MAIN CONCLUSIONs

❑ **Participation in the ILCT and Diagnostic Capacity**: Western Balkan countries, including Serbia, North Macedonia, Bosnia, Kosovo, and Montenegro, **actively participated in the XXI ILCT (2023-2024).** These countries demonstrated **adequate diagnostic capabilities** by employing both serological and virological (PCR tests) methods. All countries have the **required minimum standards for ASF antibody and genome detection techniques**.

❑ **Provision of Training and Resources:** EURL has provided **hands-on support**, including **training and the provision of biological materials** which have helped them enhance their diagnostic capabilities.

NATIONAL REFERENCE LABORATORIES (NRLs)

SEROLOGICAL TECHNIQUES

VIROLOGICAL TECHNIQUES

MAIN CONCLUSIONs

European Union Reference Laboratory for African Swine Fever

❑ **Support in ASF Outbreak Diagnosis**: The EURL has provided support in the **confirmatory diagnosis of ASF outbreaks in Serbia, North Macedonia, Bosnia, and Montenegro**. In **2024**, the cooperation **has extended to Kosovo and Albania** to further enhance regional collaboration in ASF control efforts.

❑ **Tracing the outbreaks:** Genetic characterization of ASF strains from the region has clustered them within **genotype II, genetic group 19, circulating in the central-south European countries**. To continue with the genetic characterization is crucial for tracking outbreaks and ensuring coordinated transboundary efforts in ASF control.

In conclusion the **cooperation between the Western Balkan countries and the EURL** has improved the ASF diagnostic capacity. This partnership will continue to be crucial in addressing future ASF outbreaks and ensuring the region's preparedness to combat this disease effectively.