

anses



French agency for food, environmental
and occupational health & safety

Investigate, evaluate, protect



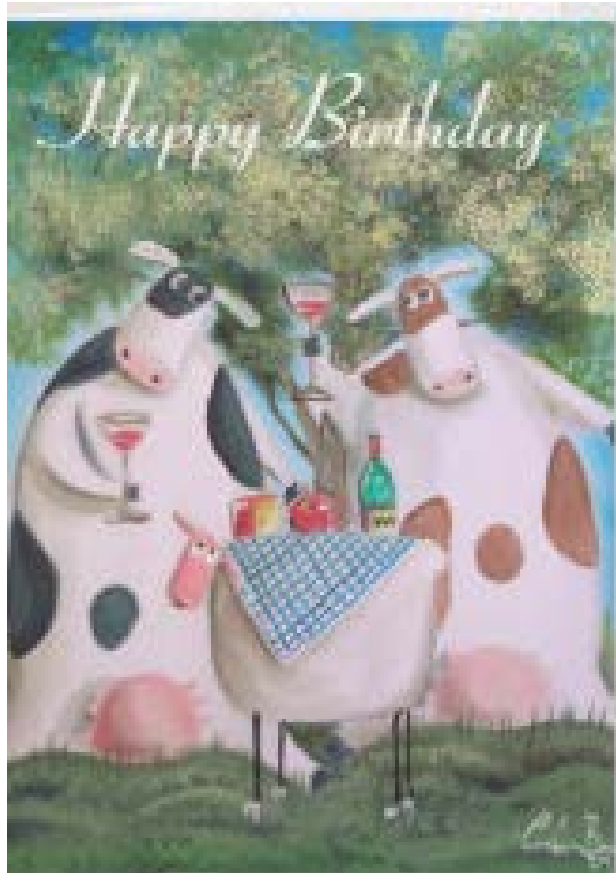
New approaches to fill surveillance gaps in West Africa

***Aurore Romey, Anthony Relmy, Kamila Gorna, Eve Laloy, Stéphan Zientara,
Sandra Blaise-Boisseau and Labib Bakkali-Kassimi***

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Towards global control and eradication of FMD

- FMD still endemic in several area of the world
- Control of the disease requires implementing adequate control measures based on risk assessment and risk based control strategies

NEEDS

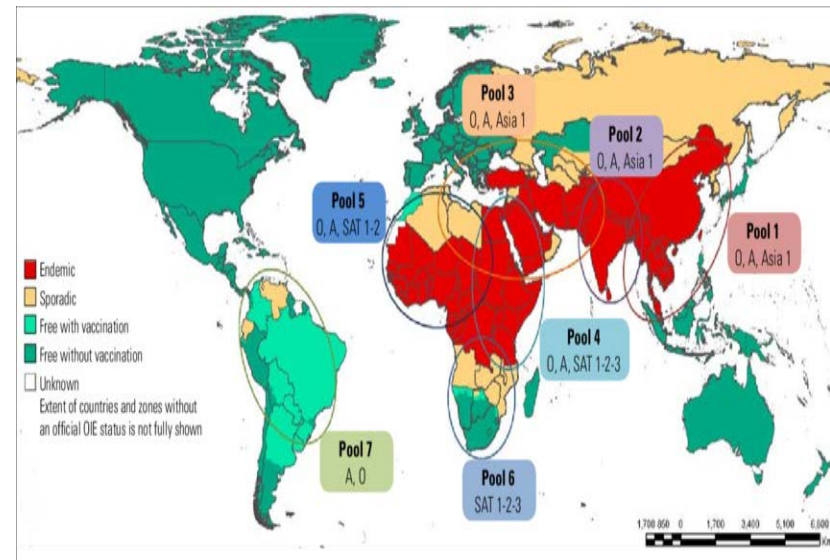


- Identification of circulating virus strains
- Understanding the dynamics of the virus

REQUIRES

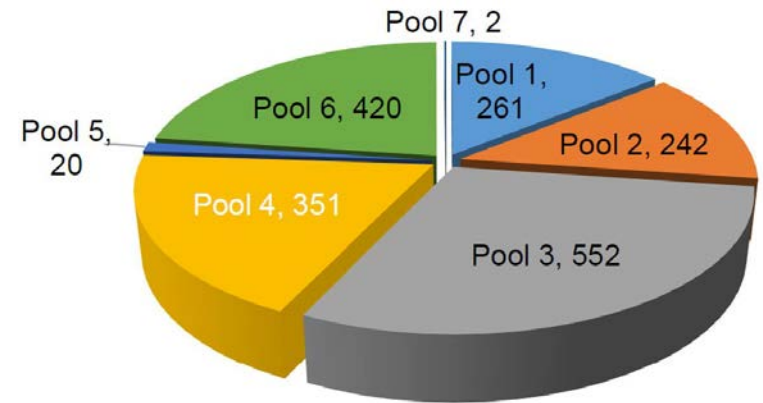
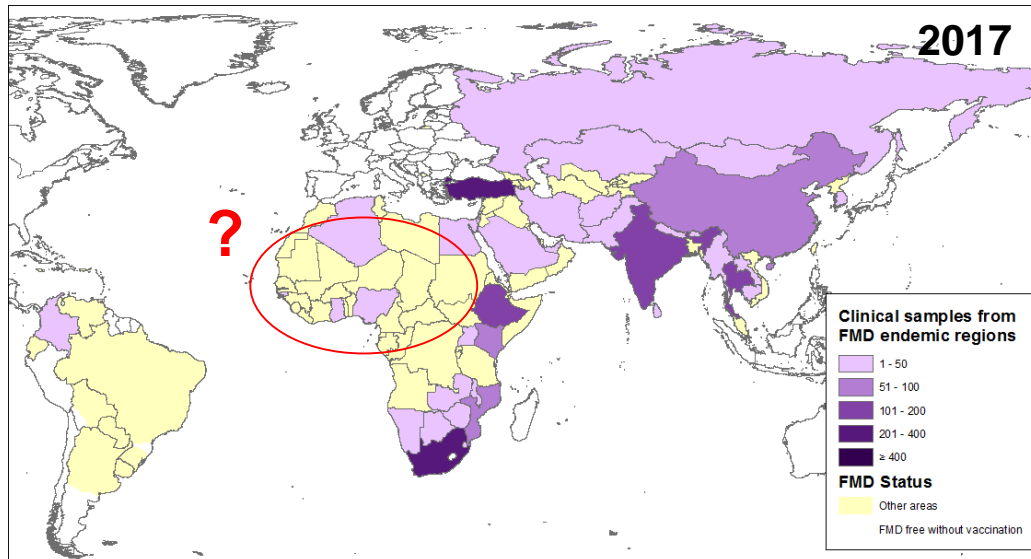


Regular submission of samples to reference laboratories for virus characterisation



Freimanis et al, 2016

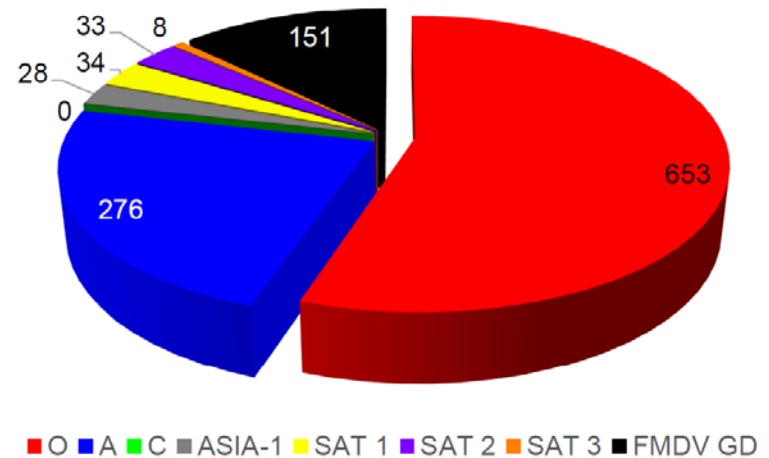
Samples submitted to OIE/FAO RLs in 2017



Few or no samples submitted from endemic countries in West and Central Africa

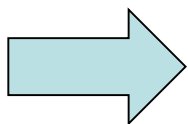
Figure 2-4: Distribution of samples collected from suspect cases of FMD (highlighted in purple) and tested by the OIE/FAO FMD Laboratory network during 2017.

Figure 2-6: Summary of results for characterised isolates (n=1183) from FMD endemic countries were reported by the Network during 2017. FMDV GD denotes samples that were only positive using molecular (RT-PCR methods), while a further 674 samples were tested but found to be negative for FMDV using all diagnostic methods.



OIE/FAO Reference Laboratory
Network for Foot-and-Mouth Disease

OIE/FAO FMD Laboratory Network report 2017



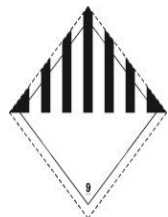
There is a need to improve regular submission of positive samples to reference laboratories



One of the main barriers



Infectious materiel



UN1845
Dry ice

Dangerous Good

high package weight and size



High Cost

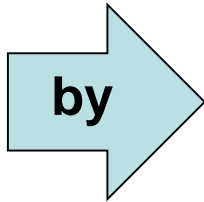


Banned by some airlines



Delay (Authorized carrier, export/import authorisations...)

How to solve these difficulties?



- ✓ Shipment of non infectious material
- ✓ Shipment under room temperature
- ✓ Test samples before shipment



eofmd
european commission for the
control of foot-and-mouth disease



2013

EUFMD – Fund for Applied
Research (EuFMD-FAR)



ORIGINAL ARTICLE
Safe and cost-effective protocol for shipment of samples from Foot-and-Mouth Disease suspected cases for laboratory diagnostic
A. Romey, A. Reimy, K. Gorna, E. Lakoy, S. Zientara, S. Blaise-Bosseau ^{1,2},
L. Bakkali Kassem
First published: 7 April 2017 Full publication history
DOI: 10.1111/tbed.12648 View/Save citation

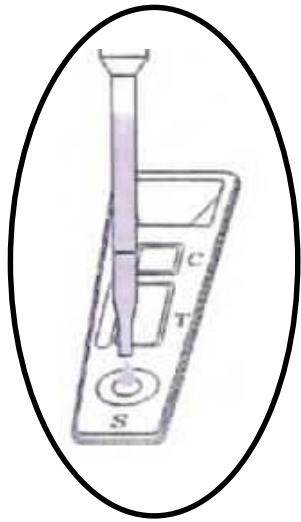
Use of lateral flow device (LFD) for safe and low cost shipment of FMDV suspected samples (FMDVINACT)



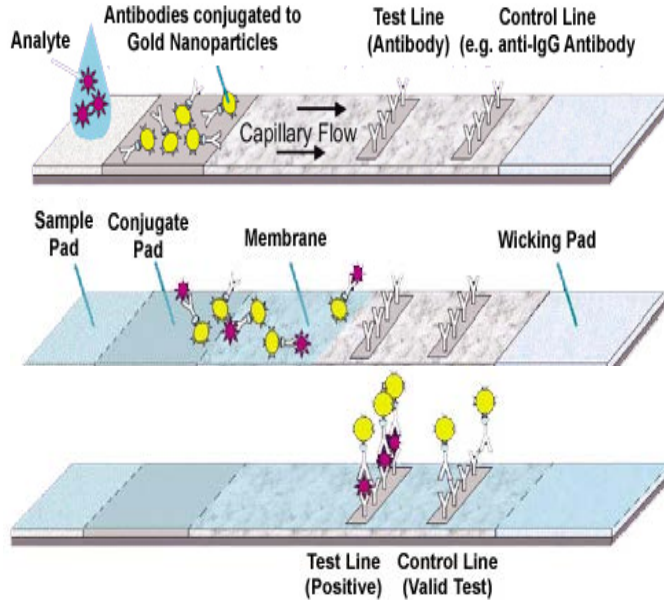
Shipment at room temperature of LFDs tested positive in the field and as non-dangerous goods after inactivation of the virus

The lateral flow device (LFD): a support for shipment

Early diagnosis method routinely used on field: immunodetection method on strip



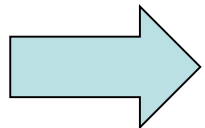
SVANODIP® FMDV-Ag
Boehringer Ingelheim Svanova



<http://www.cytodiagnostic.com>



Or

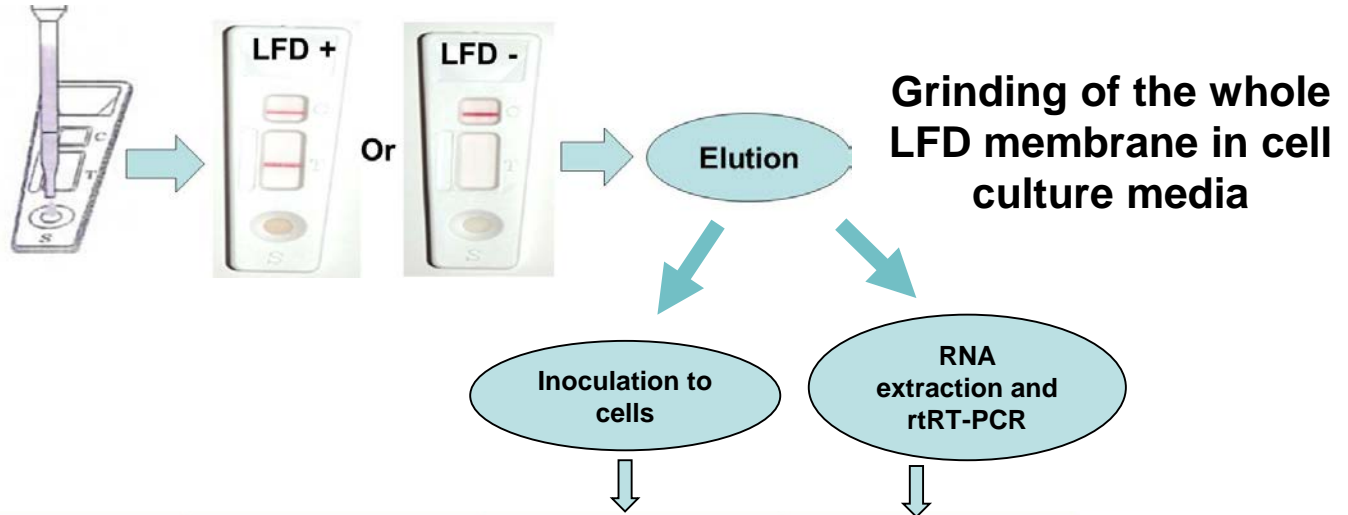


Selection and shipment of positive LFDs

The virus remains alive in the LFD



FMDV O Manisa
Different quantities



Viral load (TCID ₅₀)	LFD result	Viral isolation (on IBRS-2 and ZZ-R127)	Real time RT-PCR 3D (Ct values)
2000	Positive	CPE 18 hpi	20.33
200	Doubtful	CPE 24 hpi	23.64
20	Negative	CPE 30 hpi	27.36

How to inactivate the virus ?

FMDV is sensitive to pH → Inactivation with Citric acid & Sodium hydroxide

Mix 160µl virus + 160µl solution → 15mn incubation at RT → Inoculation to cells

Assays	Virus titer FMDV O/IRN/13/2012	Cell line	C ₆ H ₈ O ₇ (%)			NaOH (%)	
			0.3	0.2	0.1	0.2	0.1
Assay 1	10 ^{6.36} TCID ₅₀ /ml	ZZ-R-127	Tx	-	CPE	Tx	CPE
		IBRS-2	Tx	-	CPE	Tx	-
Assay 2	10 ^{6.09} TCID ₅₀ /ml	ZZ-R-127	Tx	-	CPE	Tx	CPE
		IBRS-2	Tx	-	CPE	Tx	-

Tx = toxicity effect

- = no toxicity and no cytopathic effect

CPE = cytopathic effect



0.2% citric acid solution completely inactivates FMDV O in solution in 15mn

What is the minimum incubation time needed ?

160µl virus + 160µl C₆H₈O₇ 0.2% → incubation at RT at different times → Inoculation to cells

Assays	Virus titer FMDV O Manisa	Time of contact between live virus and C ₆ H ₈ O ₇ 0.2%									
		15s	30s	1mn	2mn	4mn	6mn	8mn	10mn	12mn	15mn
Assay 1	10 ^{6.85} TCID ₅₀ /ml	CPE	CPE	-	-	-	-	-	-	-	-
Assay 2	10 ^{4.95} TCID ₅₀ /ml	CPE	-	-	-	-	-	-	-	-	-

- = no toxicity and no cytopathic effect

CPE = cytopathic effect

➔ **1 min incubation with 0.2% C₆H₈O₇ solution is sufficient to inactivate FMDV O in solution.**

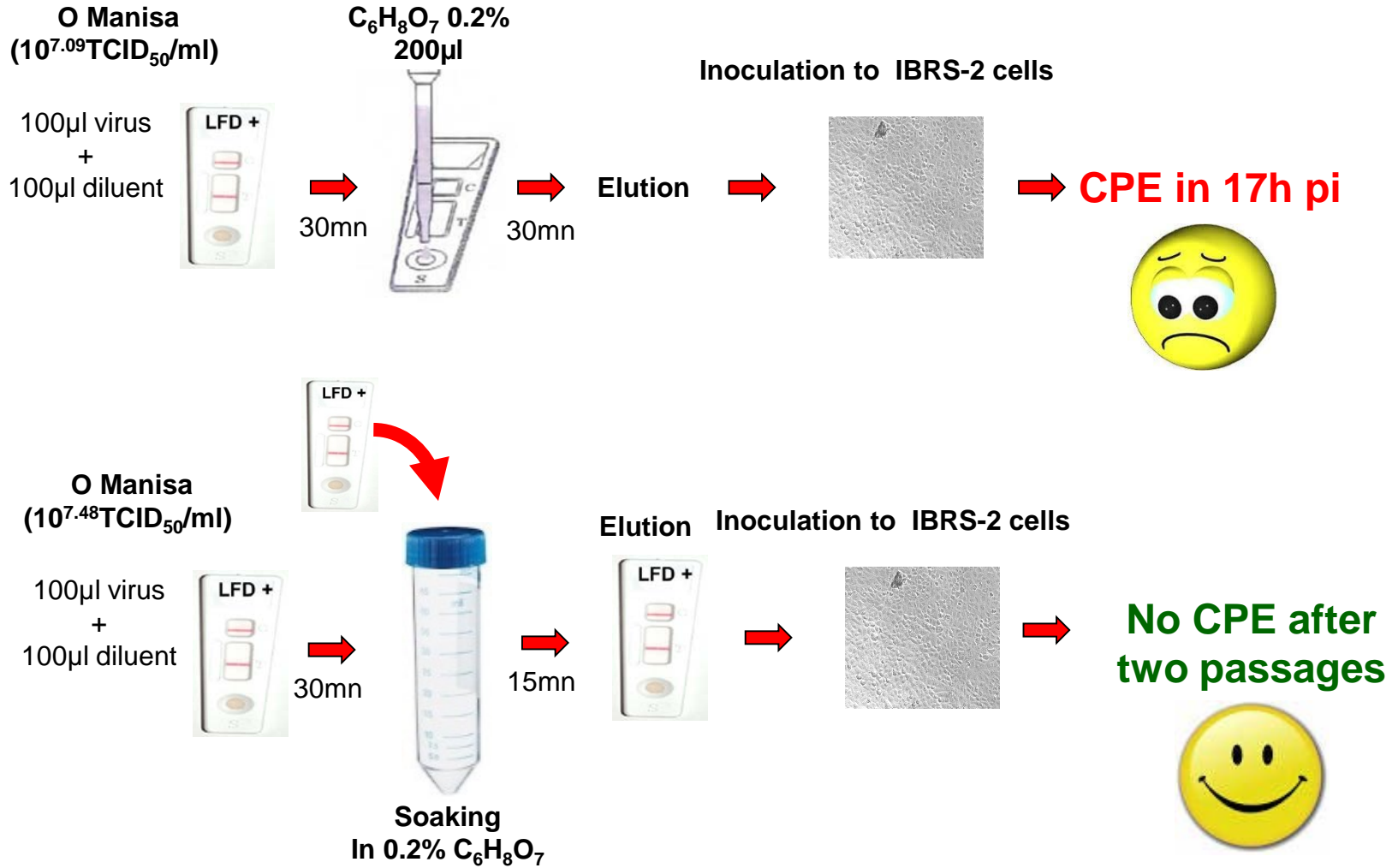
To increase safety, we chose 15 min incubation time.

A 15 minutes-contact time inactivates different FMDV serotypes in solution while the 3D coding region is still detected by rtRT-PCR

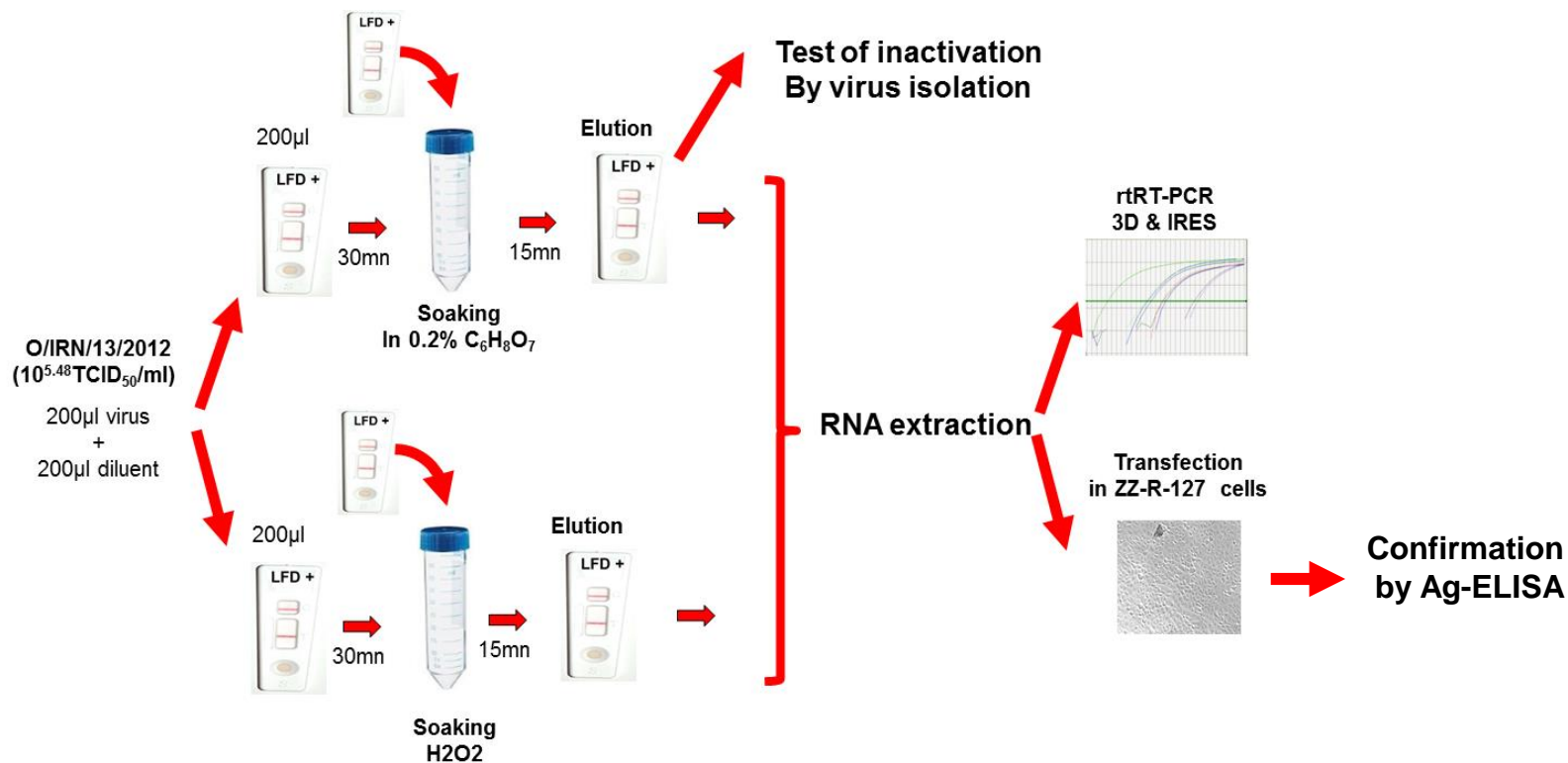
Strains	Virus titers (TCID ₅₀ /ml)	Live virus rtRT-PCR 3D Ct	Inactivated virus rtRT-PCR 3D Ct	CPE on cells (ZZ-R-127 & IBRS-2) after 2 nd passage
O Manisa TUR/8/69	10 ^{6.72}	16.64	18.60	-
O1 BFS 1860	10 ^{7.99}	12.94	14.29	-
OMayenne (O/FRA/1/2001)	10 ^{7.36}	13.19	14.19	-
O/IRN/13/2012	10 ^{7.48}	13.29	13.27	-
A5 Allier	10 ^{5.95}	15.30	14.77	-
A22Iraq	10 ^{6.72}	17.28	17.09	-
A24Cruzeiro	10 ^{6.95}	15.08	16.04	-
Alran96	10 ^{6.95}	18.33	17.52	-
A/IRN/37/2009	10 ^{6.23}	15.24	15.31	-
A/IRN05	10 ^{7.23}	15.04	14.68	-
C1 Noville	10 ^{8.15}	14.09	13.99	-
SAT1/KEN/2/2011	10 ^{5.82}	13.11	13.68	-
SAT2/ZIM/5/81	10 ^{7.23}	17.77	17.38	-
SAT2/EGY3/2012	10 ^{7.69}	22.07	22.08	-
SAT2/LIB40/2012	10 ^{7.72}	13.79	13.50	-
SAT2/BAR 12/2012	10 ^{7.48}	11.36	11.83	-
SAT2/ERI	10 ^{5.72}	13.41	13.96	-
SAT3 Zim 4/81	10 ^{6.95}	16.63	16.42	-
Asia/ISR/3/89	10 ^{7.15}	15.38	16.78	-

- = no toxicity and no cytopathic effect

Inactivation of live FMD virus on LFD



Detection of FMDV RNA and rescue of live virus after inactivation?



Samples	3D Ct values	IRES Ct values	Transfection ZZ-R-127
LFD without inactivation	15.41	17.33	Total CPE at less than 18hpt
LFD soaked in 0.2% $\text{C}_6\text{H}_8\text{O}_7$	17.48	17.10	Total CPE at less than 18 hpt

Inactivation and detection of FMDV RNA and rescue of live virus for other serotypes

Strains	Virus titers (TCID ₅₀ /ml)	LFD result*	Dipping solution	CPE on cells after elution	3D Ct	IRES Ct	CPE on cells after RNA transfection
A/IRN05	10 ^{7.48}	++	Water	++ 24 hpi	19.60	21.76	++ 24 hpt
			C ₆ H ₈ O ₇ 0.2 %	-	19.15	20.46	++ 48 hpt
C1 Noville	10 ^{7.72}	+++	Water	++ 24 hpi	18.01	24.01	++ 24 hpt
			C ₆ H ₈ O ₇ 0.2 %	-	17.29	23.21	++ 48 hpt
SAT1/KEN/2/2011	10 ^{5.82}	++	Water	++ 5 hpi	18.48	21.42	++ 24 hpt
			C ₆ H ₈ O ₇ 0.2 %	-	16.91	20.71	++ 24 hpt
SAT2/LIB40/2012	10 ^{8.36}	+	Water	++ 24 hpi	14.12	39.37	++ 24 hpt
			C ₆ H ₈ O ₇ 0.2 %	-	12.73	37.75	++ 24 hpt
SAT3 Zim 4/81	10 ^{6.95}	++	Water	++ 5 hpi	19.75	29.49	++ 24 hpt
			C ₆ H ₈ O ₇ 0.2 %	-	18.24	26.88	++ 24 hpt
Asia/ISR/3/89	10 ^{6.69}	+	Water	++ 24 hpi	30.42	29.44	++ 48 hpt
			C ₆ H ₈ O ₇ 0.2 %	-	30.76	26.61	++ 48 hpt

*LFD positive results: +++ = strong, ++ = intermediary, + = weak

- = no cytopathic effect after two passages on cells

hpi: hours post-inoculation

hpt: hours post-transfection

Application of inactivation method on archival field samples

Sample	Virus titre (TCID ₅₀ /ml)	LFD result ^a	Soaking solution	CPE on cells after inoculation	3D Ct	IRES Ct	VP1 sequence homology ^b	CPE on cells after RNA transfection
FMDV/TUN/1771/2014	10 ^{5.95}	+	H ₂ O	+24 hpi	25.56	NA	100%	+24 hpt
			C ₆ H ₈ O ₇ 0.2%	-	25.00	NA		+24 hpt
BEN/1/2011	10 ^{3.48}	+	H ₂ O	+48 hpi	25.41	36.46	100%	+48 hpt
			C ₆ H ₈ O ₇ 0.2%	-	23.58	33.14		+48 hpt
O/FRA/DPT77/2001	10 ^{4.23}	+++	H ₂ O	+48 hpi	19.98	21.45	100%	-
			C ₆ H ₈ O ₇ 0.2%	-	20.23	20.95		-

FMDV, foot-and-mouth disease virus; LFD, lateral flow device; -, no cytopathic effect after two passages on cells; hpi, hours post-inoculation; hpt, hours post-transfection; NA, not applicable.

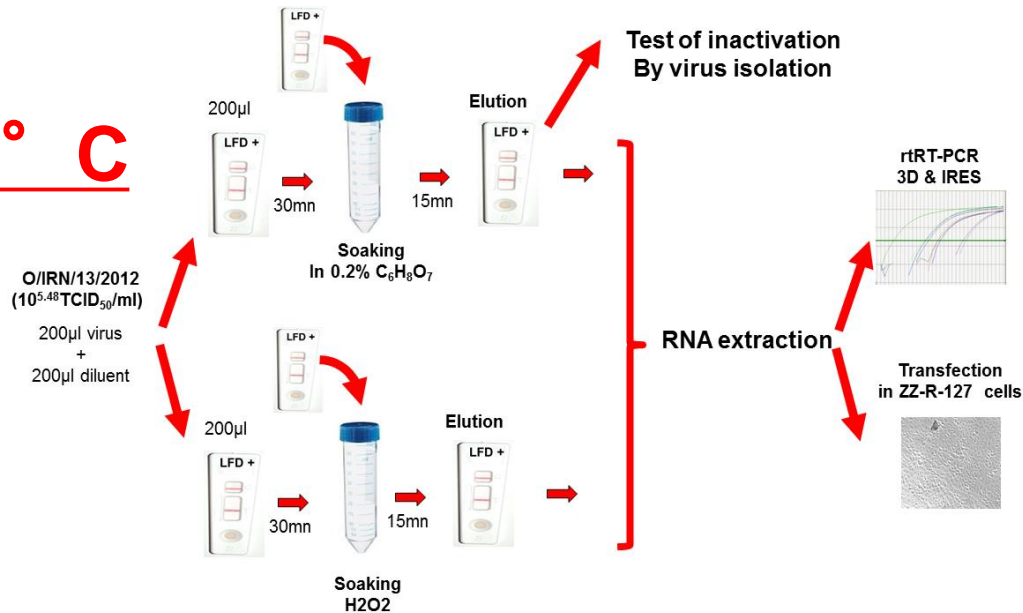
^a+++ = strong, + = weak.

^bBased on comparison of the 639 bp of the serotype O VP1.

- ➔ The inactivation protocol is applicable on field samples: virus eluted from inactivated LFD can be still detected and characterized.
- ➔ Recovery of live virus after chemical transfection was obtained for 2/3 samples (protocol needs improvement).

Inactivation at 37°C and inactivation with 5% C₆H₈O₇

RT or 37° C



Dipping solution	Temperature	CPE on cells after soaking step	3D Ct	IRES Ct	CPE on cells after RNA transfection
Water	RT	++ 24 hpi	19.45	22.05	++ 48 hpt
Water	37 ° C	++ 24 hpi	20.62	22.97	++ 48 hpt
C ₆ H ₈ O ₇ 0.2 %	RT	-	20.65	17.53	++ 48 hpt
C ₆ H ₈ O ₇ 0.2 %	37 ° C	-	18.40	20.18	++ 48 hpt
C ₆ H ₈ O ₇ 5 %	RT	-	17.39	17.45	++ 48 hpt

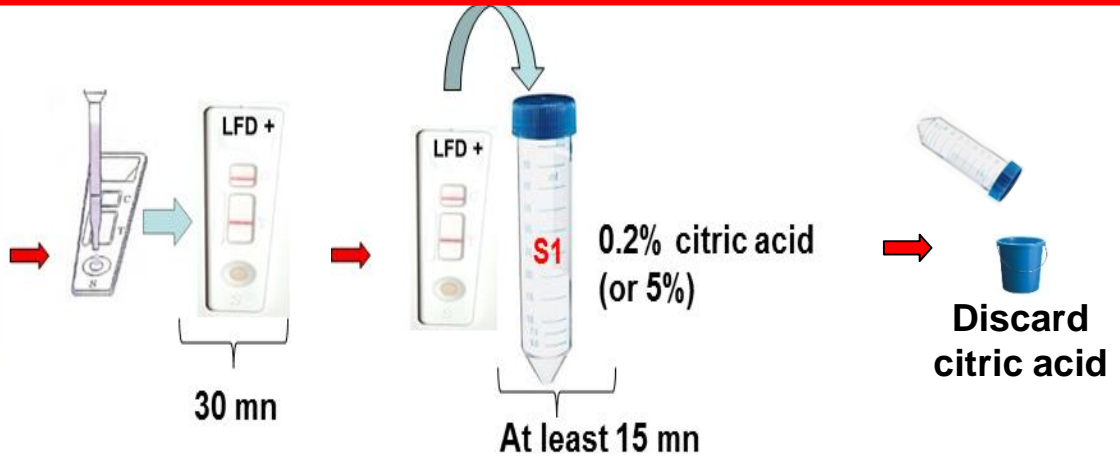
- = no cytopathic effect after two passages on cells

hpi: hours post-inoculation

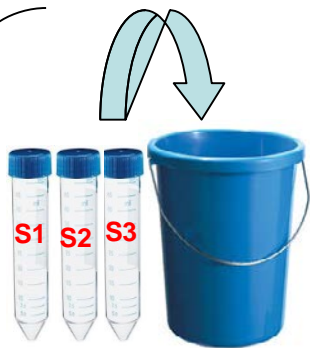
hpt: hours post-transfection

Example of procedure to apply on field...

In farm

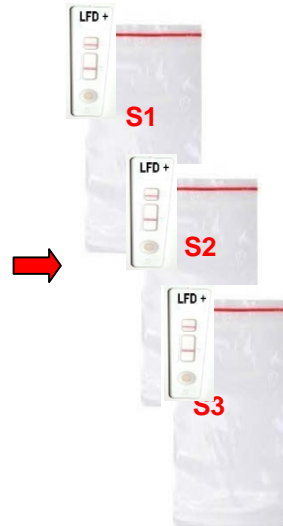


In decontamination area



Decontamination
solution

In clean area



Triple packaging

At room T°



Towards approval of biosafety and application.....



Still some issues to address:

- Improvement of RNA transfection;
- Validation of the protocol on the field with fresh samples;
- Testing the efficacy of inactivation on highly concentrated virus (Vesicular fluid)



EUFMD – Fund for Applied Research (EuFMD-FAR) - 2017

Evaluation in field conditions of a safe and cost-effective protocol for shipment of samples from FMD suspected cases for laboratory diagnostic (FIELD_EVAL_INACT)

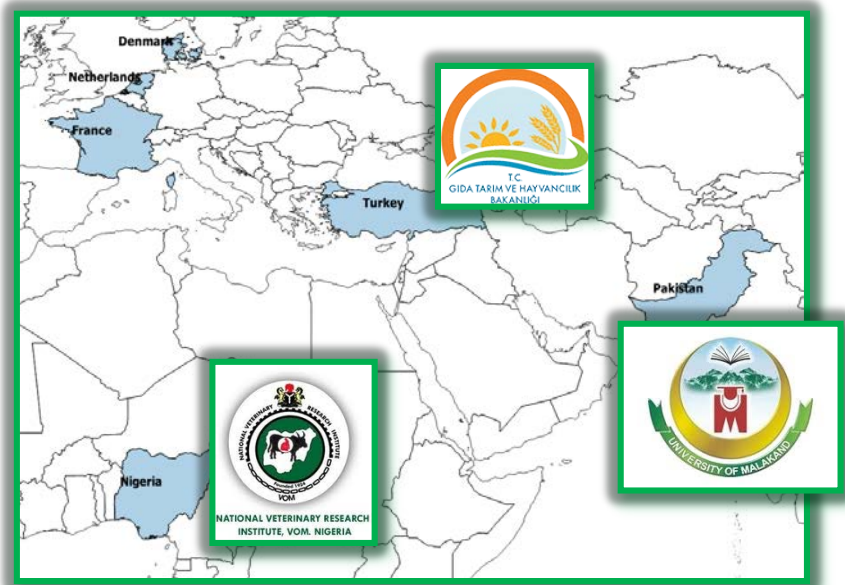
- Anses, France (coordinator)
- Technical University of Denmark (DTU)
- FMD Research Centre of Nigeria (NRVI)
- FMD Institute of Turkey (SAP)
- University of Malakand in Pakistan (UM)
- Merial- Boehringer Ingelheim (BI)



Samples collection and inactivation of LFD in the field



Ularanu Hussaini, Nigeria, 2018



Ularanu Hussaini, Nigeria, 2018



Naci Bulut, Turkey, 2018



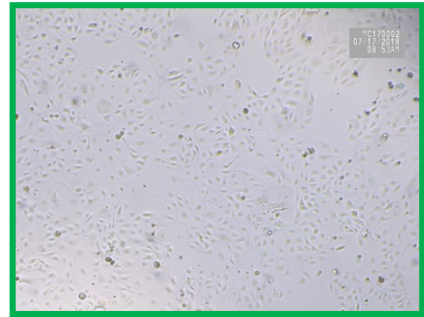
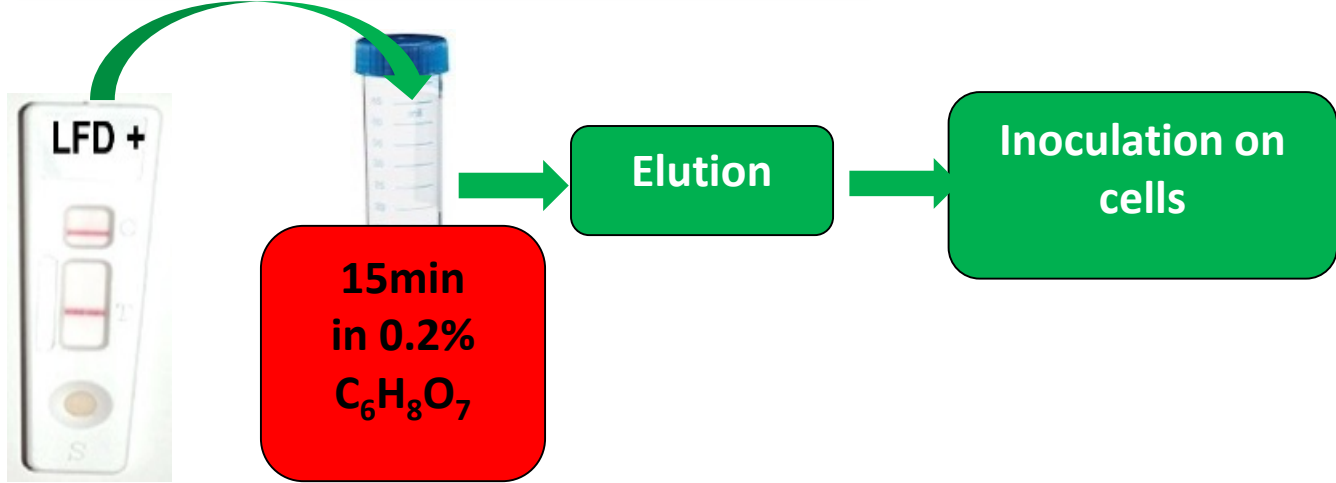
Naci Bulut, Turkey, 2018

Ularanu Hussaini, Nigeria, 2018

Safety tests in the lab



France
Nigeria
Turkey
... in progress



Molecular detections in the lab

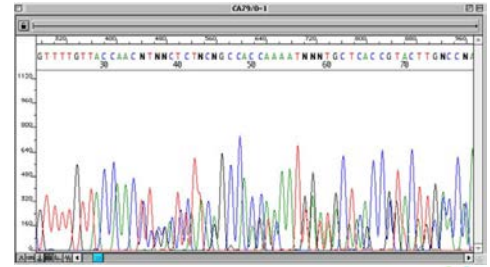
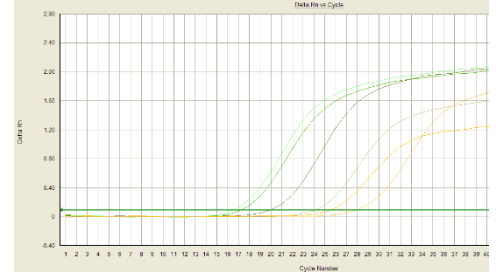


15min
in 0.2%
 $C_6H_8O_7$

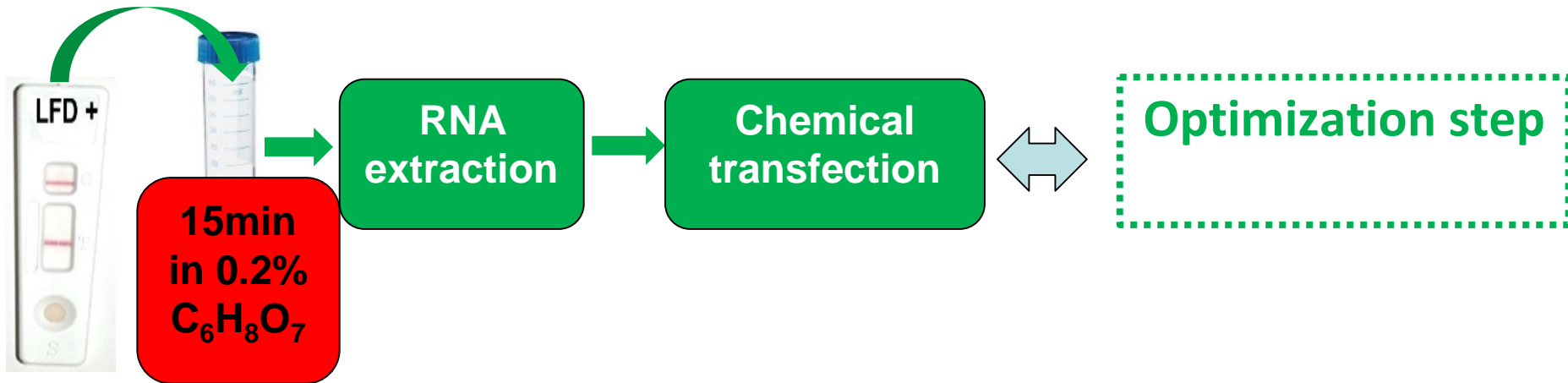
RNA
extraction

rtRT-PCR 3D
&
IRES

RT-PCR VP1 &
sequencing



Virus rescue in the lab



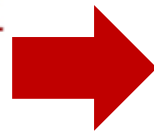
Application in the field



2018

6th Call EUFMD – Fund for Applied Research
(EuFMD-FAR) – 2018

Pilot studies – West /Central Africa



Pilot study in Mali

Support



Boehringer
Ingelheim

- 1. Establish the feasibility of engaging paraveterinarians, private animal health service providers or other non-state actors in FMD sample collection and submission to the national laboratories/authorities;**
- 2. A study on the demand of livestock keepers and other stakeholders for services for prevention or management of FMD, to establish if a market potential exists for services (including early warning of risk) and which will identify what will need to change if the demand is to be met and/or the service to be introduced**

Application of inactivation protocol in Mali



CONCLUSION

- 15 min incubation in 0.2% citric acid is sufficient for inactivation of FMDV on LFD
- FMDV RNA can be extracted from LFD and FMDV detected by rtRT-PCR, VP1 sequenced and live virus rescued after RNA transfection
- Validation of the protocol on the field is ongoing (FAR 2017 & 2018)
- The protocol should facilitate the transport of samples and thus increase the submissions
- The protocol needs to be evaluated and validated by the Biorisk Working Group of the EuFMD

Acknowledgement



NATIONAL VETERINARY RESEARCH
INSTITUTE, VOM, NIGERIA



FRIEDRICH-LOEFFLER-INSTITUT



Thank you for your attention

