

# Use of lateral flow device for safe and low cost shipment of FMDV suspected samples

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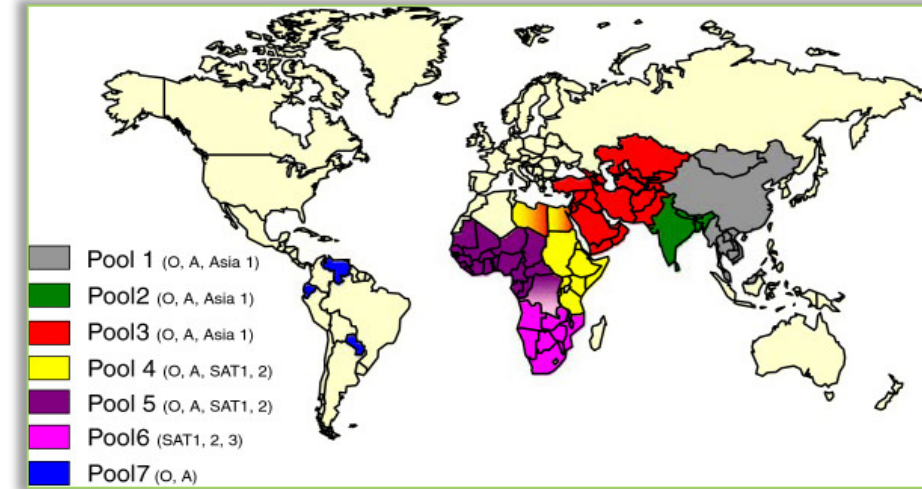


## Towards global control & eradication of FMD...

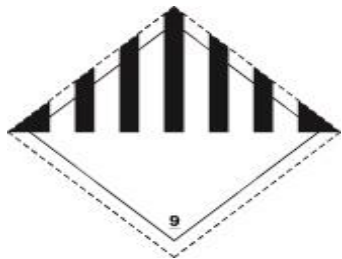
- ✓ To identify infected regions
- ✓ To identify circulating strains
- ✓ To study virus dynamics

## But in endemic areas...

- ✓ Lack of FMD laboratory diagnostic capacity
- ✓ Low or no submission of infected samples to reference laboratories



Jamal & Belsham 2013



UN1845  
(dry ice)



UN2900  
UN3373



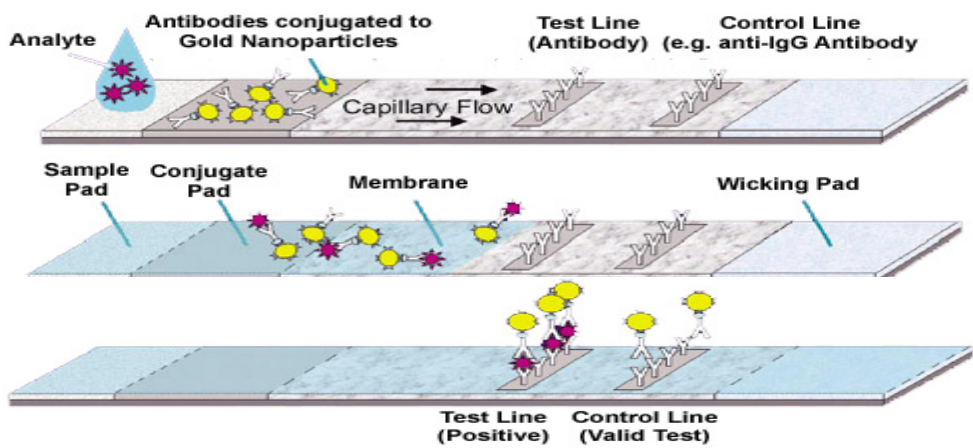
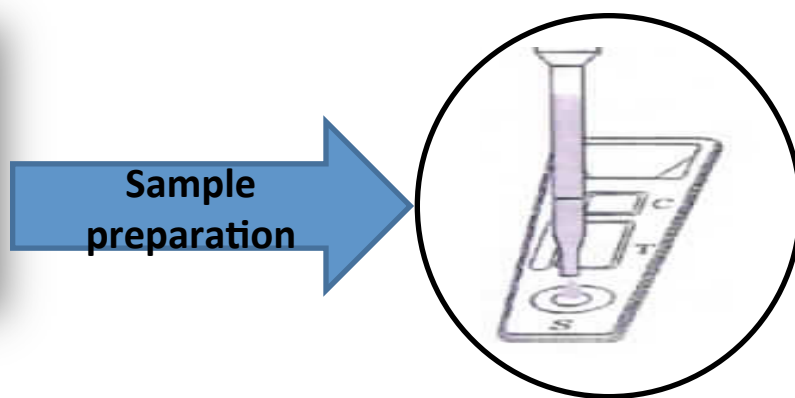


To develop a low cost and safe method for shipment of FMDV suspected samples based on the use of...

Lateral Flow Device (LFD) SVANODIP® FMDV-Ag (Boehringer Ingelheim Svanova)



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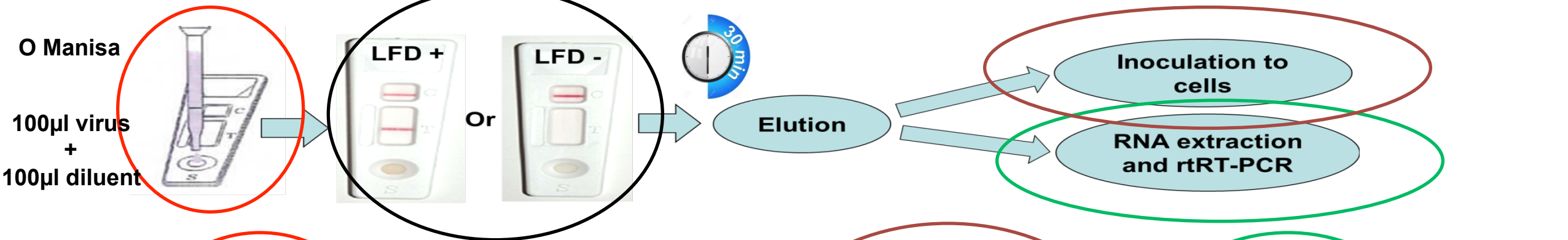


<http://www.cytodiagnostic.com>

- ✓ Immunochromatographic strips
- ✓ Penside detection of FMDV Ag on field
- ✓ Sample= vesicular fluid or lesion epithelium
- ✓ Accurate result after 10 min

**Safety of positive LFD for shipment ???**

## Live FMDV eluted from positive LFDs ?

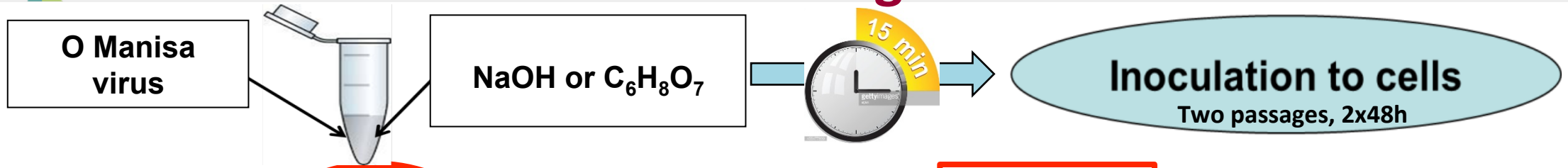


Viral load (TCID <sub>50</sub> )	LFD result	Viral isolation (on IBRS-2 and ZZ-R127)	Real time RT-PCR 3D (Ct values)
2000	Positive	CPE 18 hpi	20.33
200	Doubtful	CPE 24 hpi	23.64
20	Negative	CPE 30 hpi	27.36

- ➔ Infectious virus (Type O) is eluted from loaded LFDs (positive, doubtful even negative)
- ➔ Live FMDV still recovered 18h, 7 days and 14 days after LFD loading (positive LFDs stored at RT)

**Inactivation of LFDs loaded with FMDV is required to ensure safe shipment.**

# Choice of the inactivating solution



Assays	Virus titer	Cell line	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> (%)			NaOH (%)	
			0.3	0.2	0.1	0.2	0.1
Assay 1	10 <sup>6.36</sup> TCID <sub>50</sub> /ml	ZZ-R-127	Tx	-	CPE	Tx	CPE
		IBRS-2	Tx	-	CPE	Tx	-
Assay 2	10 <sup>6.09</sup> TCID <sub>50</sub> /ml	ZZ-R-127	Tx	-	CPE	Tx	CPE
		IBRS-2	Tx	-	CPE	Tx	-

Tx = toxicity effect

CPE=cytopathic effect

- = no toxicity and no cytopathic effect

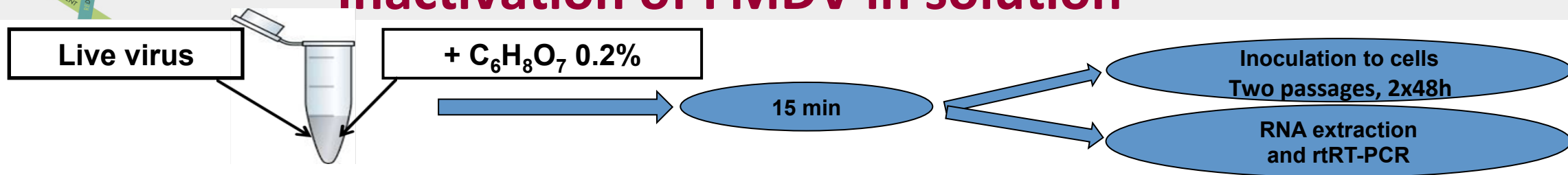
**0.2% citric acid solution completely inactivates FMDV in solution in 15 min.**

Assays	Virus titer	Time of contact between live virus and C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> 0.2 %									
		15sec	30sec	1min	2min	4min	6min	8min	10min	12min	15min
Assay 1	10 <sup>6.85</sup> TCID <sub>50</sub> /ml	CPE	CPE	-	-	-	-	-	-	-	-
Assay 2	10 <sup>4.95</sup> TCID <sub>50</sub> /ml	CPE	-	-	-	-	-	-	-	-	-

**1 min incubation with C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> 0.2% solution is sufficient to inactivate FMDV strain in solution.**



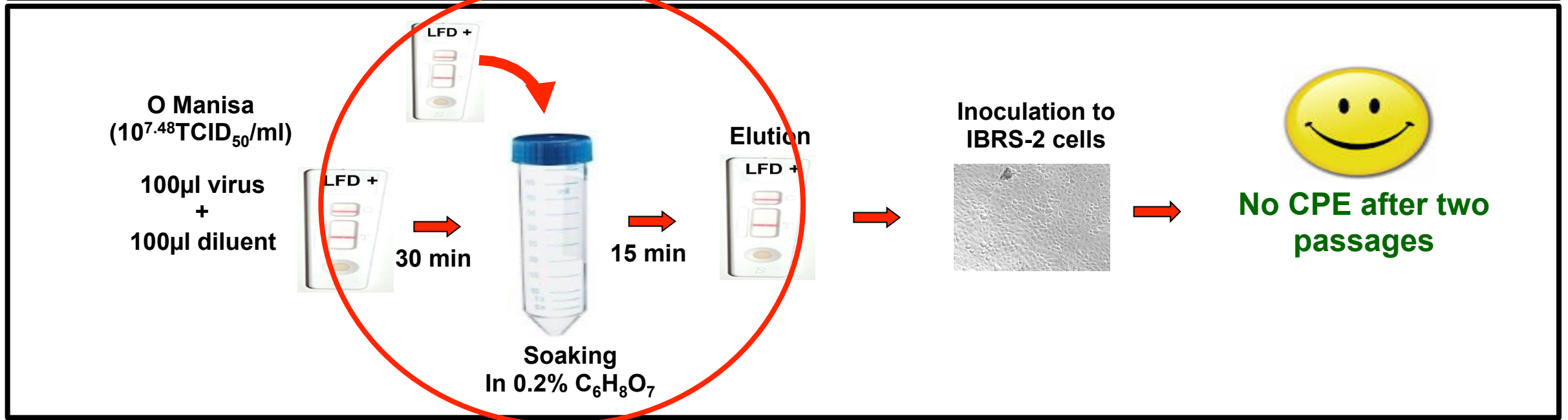
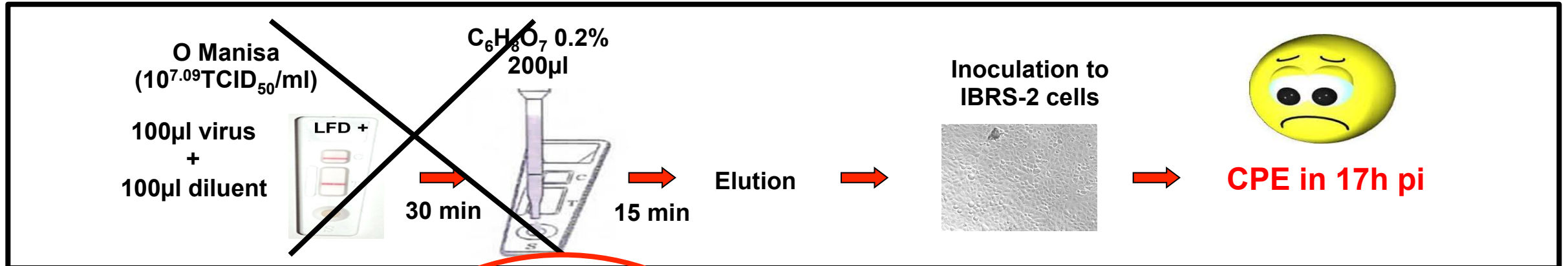
# Inactivation of FMDV in solution



Strains	Virus titers (TCID <sub>50</sub> /ml)	Live virus rtRT-PCR 3D Ct	Inactivated virus rtRT-PCR 3D Ct	CPE on cells after 2 <sup>nd</sup> passage
O Manisa TUR/8/69	10 <sup>6.72</sup>	16.64	18.60	-
O1 BFS 1860	10 <sup>7.99</sup>	12.94	14.29	-
O Mayenne (O/FRA/1/2001)	10 <sup>7.36</sup>	13.19	14.19	-
O/IRN/13/2012	10 <sup>7.48</sup>	13.29	13.27	-
<b>15 min incubation in C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> 0.2% solution is sufficient to inactivate FMDV strains representative of the 7 serotypes while the 3D coding region is still detected by rtRT-PCR</b>				
SAT1/KEN/2/2011	10 <sup>5.82</sup>	13.11	13.68	-
SAT2/ZIM/5/81	10 <sup>7.23</sup>	17.77	17.38	-
SAT2/EGY3/2012	10 <sup>7.69</sup>	22.07	22.08	-
SAT2/LIB40/2012	10 <sup>7.72</sup>	13.79	13.50	-
SAT2/BAR 12/2012	10 <sup>7.48</sup>	11.36	11.83	-
SAT2/ERI	10 <sup>5.72</sup>	13.41	13.96	-
SAT3 Zim 4/81	10 <sup>6.95</sup>	16.63	16.42	-
Asia/ISR/3/89	10 <sup>7.15</sup>	15.38	16.78	-

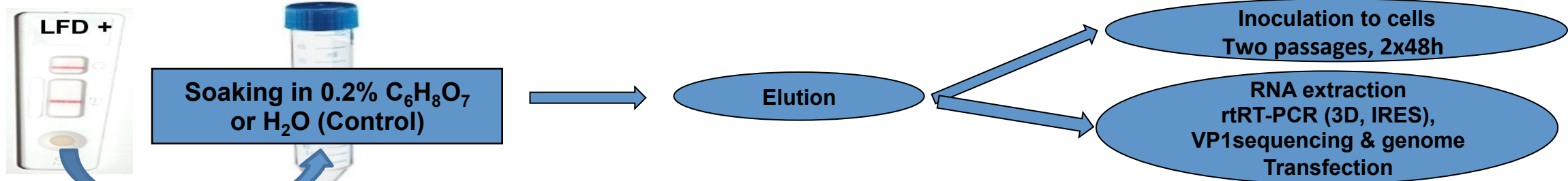
- = no toxicity and no cytopathic effect

# Inactivation of FMDV loaded on LFD



**Live virus is inactivated by soaking positive LFD in  $\text{C}_6\text{H}_8\text{O}_7$  0.2 % bath during 15 min**

# FMDV inactivation on LFD and further detection



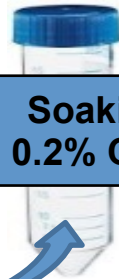
Strains	Virus titers (TCID <sub>50</sub> /ml)	LFD result*	Soaking solution	CPE on cells after inoculation	3D Ct	IRES Ct	VP1 amplification	CPE on cells after RNA transfection
O/IRN/13/2012	10 <sup>7.23</sup>	++	H <sub>2</sub> O	+24 hpi	19.41	16.50	1155 bp	+24 hpt
			C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> 0.2 %	-	20.65	17.53		+48 hpt
A/IRN05	10 <sup>7.48</sup>	++	H <sub>2</sub> O	+24 hpi	19.60	21.76	846 bp	+24 hpt
			C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> 0.2 %	-	19.15	20.46		+48 hpt
C1 <u>Noville</u>	10 <sup>7.72</sup>	+++	H <sub>2</sub> O	+24 hpi	18.01	24.01	837 bp	+24 hpt
			C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> 0.2 %	-	17.29	23.21		+48 hpt
SAT1/KEN/2/2011	10 <sup>5.82</sup>	++	H <sub>2</sub> O	+5 hpi	18.48	21.42	1023 bp	+24 hpt
			C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> 0.2 %	-	16.91	20.71		+24 hpt
SAT2/LIB40/2012	10 <sup>8.36</sup>	+	H <sub>2</sub> O	+24 hpi	14.12	39.37	1255 bp	+24 hpt
			C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> 0.2 %	-	12.73	37.75		+24 hpt
SAT3 <u>Zim 4/81</u>	10 <sup>6.95</sup>	++	H <sub>2</sub> O	+5 hpi	19.75	29.49	1254 bp	+24 hpt
			C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> 0.2 %	-	18.24	26.88		+24 hpt
Asia/ISR/3/89	10 <sup>6.69</sup>	+	H <sub>2</sub> O	+24 hpi	30.42	29.44	894 bp	+48 hpt
			C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> 0.2 %	-	30.76	26.61		+48 hpt

\*: +++ = strong, ++ = intermediary, + = weak; -: no CPE after two cell-passages; hpi: hours post inoculation; hpt: hours post transfection

(Romey et al, in preparation)

After inactivation no CPE observed, genome still detected by rtRT-PCR (3D, IRES), VP1 sequenced and virus rescued after RNA transfection



O Manisa  
virusSoaking  
In 0.2% C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>

Elution

Inoculation to cells  
Two passages, 2x48hRNA extraction  
rtRT-PCR  
Transfection

37°C vs RT

Temperature	Viral load (TCID <sub>50</sub> /ml)	CPE on cells after soaking step	rtRT-PCR		CPE on cells after RNA transfection
			3D Ct	IRES Ct	
RT	10 <sup>7.36</sup>	-	20.65	17.53	++ 48 hpt
37 °C		-	18.40	20.18	++ 48 hpt



This inactivation method is efficient at 37 °C

## Field samples available in the laboratory...

Samples	Virus titers (TCID <sub>50</sub> /ml)	LFD result	CPE on cells after soaking step	rtRT-PCR 3D Ct	rtRT-PCR IRES Ct	VP1 sequencing	CPE after transfection
Serotype O Tunisie/2014	10 <sup>5.95</sup>	+	-	25.00	40.98	+	-
Serotype O Benin/2011	10 <sup>3.48</sup>	+	-	23.58	33.14	+	+

Serotype O France/2009

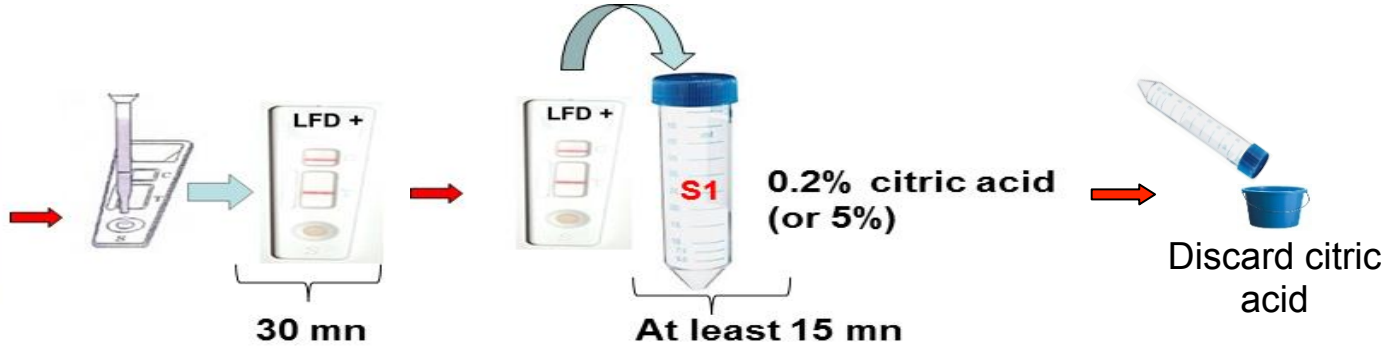
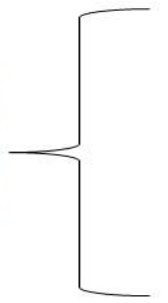
Serotype O Kenya/2015

Samples

- ✓ Results consistent with those obtained with viral strains
- ✓ Inactivation OK, Genome detection OK for all samples
- ✓ Virus rescued for 2/3 field samples in laboratory conditions
- ✓ VP1 sequenced for 3/3 field samples in laboratory conditions

Serotype O Kenya/2015	Not Done	+/+	- / -	28.21/31.30	28.12/30.26	Note Done	-
Serotype A Kenya/2015	Note Done	++/++	- / -	27.65/25.44	27.17/26.61	Note Done	-
Serotype SAT2 Kenya/2015	Note Done	+++ / +++	- / -	21.63/29.00	29.38/29.91	Note Done	-

### In farm



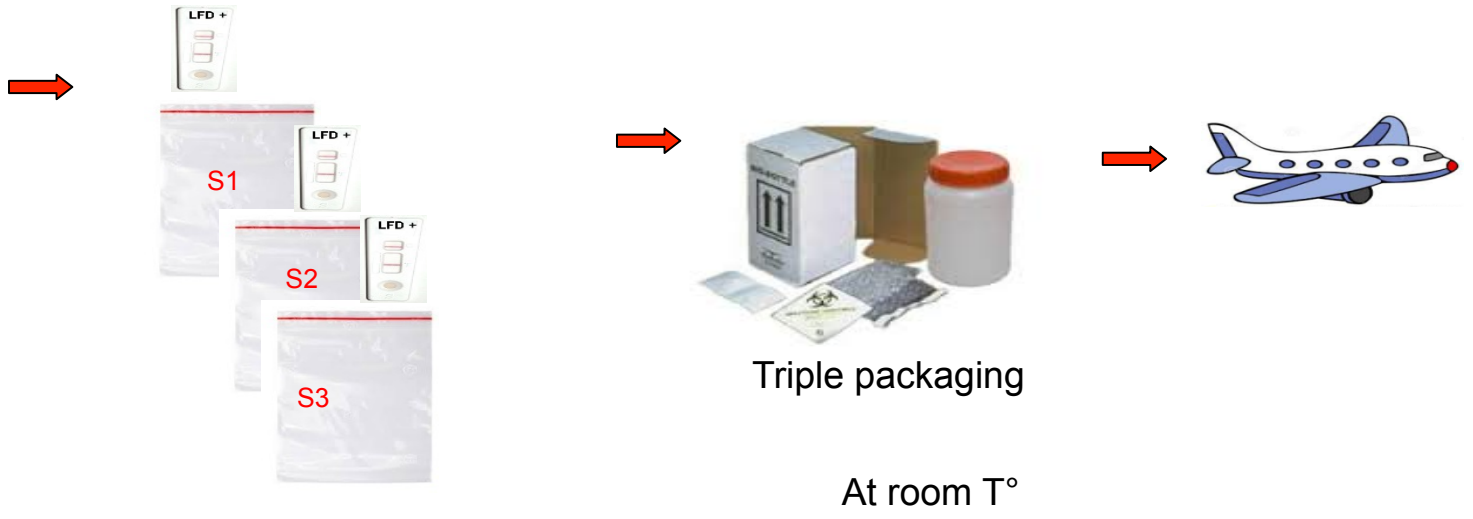
### In Decontamination area



Decontamination solution



### In Clean area



## Conclusion

- ✓ A protocol developed for low cost and safe shipment of FMDV suspected samples
- ✓ Evaluated on reference strains and 9 field samples
- ✓ A procedure proposed for collection then safe shipment of samples from field to reference laboratory

## Perspectives

- ✓ To evaluate the protocol on a larger panel of field samples
- ✓ To experience the procedure on field
- ✓ Collaborations welcomed!!

## « BIOPIC » team



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**FMDVINACT** project  
Funding by **EU**FMD



**Stephan Zientara**



**École nationale vétérinaire d'Alfort**



alimentation, environnement, travail





*And...*  
*Thank you for your attention...*

