

## "RAPIDIA-FIELD"

# (Rapid Field Diagnostics and Screening in Veterinary Medicine)

## Project objectives and achievements



Martin Beer, Scientific Coordinator

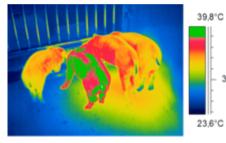
## **Diagnosis of animal diseases**





#### Detection of abnormalities

- Fever
- Reduced feed intake
- Huddling
- Depression



- Influenza
- Classical swine fever
- African swine fever
  - •••





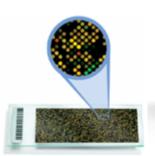
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### Testing in the field - "Pen-site"



#### Use of reference techniques





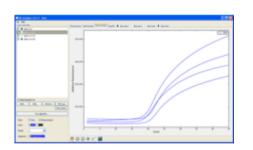
### Testing in basic laboratories

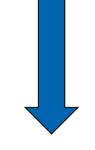


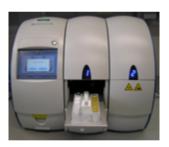
- Shipment
- Sample processing
- Testing
- Storage
- Remit to reference laboratory



Development and implementation of reliable and robust diagnostic tools as a prerequisite for the efficient and succesful control of animal diseases







Wherever possible: (validated) commercial products





# KBBE.2011.1.3-02:

# Development of field test for rapid screening of pathologies as well as simple laboratory test in animals



**CODA - CERVA** 



HEALTHY ANIMALS, SAFE FOOD.





Collaborative project: 12 partners\* Duration: 42 month Budget: 4.3 M€ (3 M€ EU contribution) Coordinator: INGENASA (Paloma Rueda) Scientific Coordinator: FLI (Martin Beer)









VETERINÄRMEDICINSKA

TATENS

ANSTALT







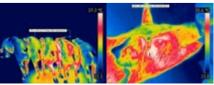
# WP2: Real-time monitoring livestock on line





## Early warning non-invasive health monitoring

- Most infectious diseases will be accompanied by alterations of body temperature, behaviour, and feed intake
- These parameters can be used as sensitive indicators for the detection of abnormalities
- Computer-aided online systems are the method of choice for industrialized holdings
- Non-invasive thermography could be applied also to backyard settings
- RAPIDIA achievements: design and validation of the RTMS-ON system (real-time monitoring system online)
- Assays: Temperature and motion monitoring or radio-frequency identification



## Real-time monitoring system online







- The technique is intended to detect the early stages of infeciton in sentinel animals by measuring physical or physiological changes through *in-vivo* sensors.
- The information is then remotely transmitted in realtime and an alert is issued when a certain threshold is reached
- less invasive than continous sampling
- real-time transmission of data
- High sensitivity to detect early changes such as increased temperature, reduced water consumption, and decrease in motion

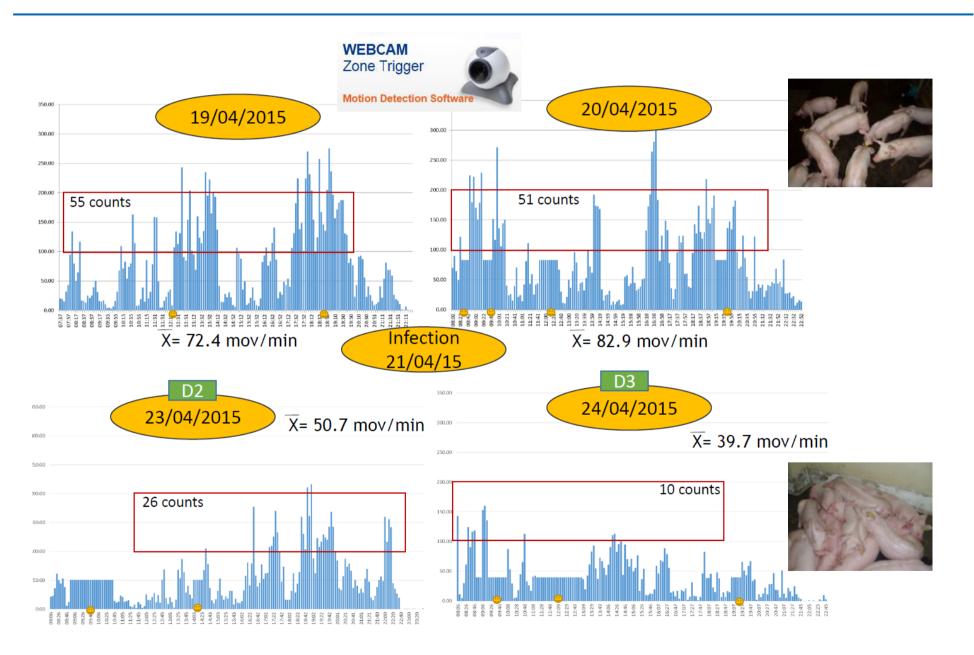


Fig. 1. Schematic diagramme of the Real-Time Monit M. Martínez-Avilés, E. Fernández-Carrión, J. M. López García-Baones and J. M. Sánchez-Vizcaíno

Visavet Centre and Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Madrid, Spain

## **Detection of motion upon ASFV infection**











# Early detection - alternative samples







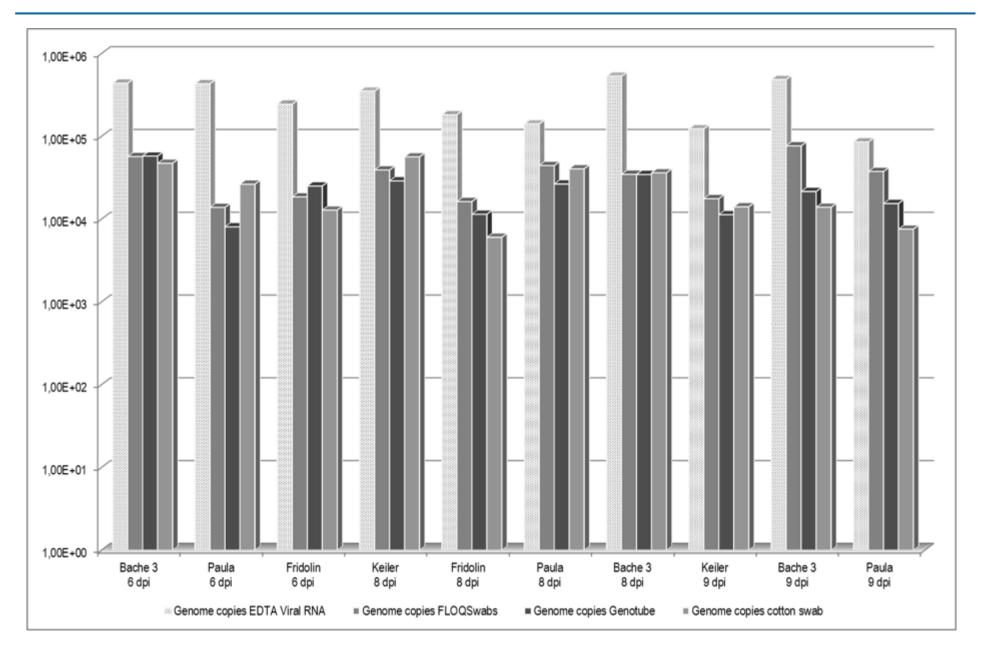
# Experiments

- Three different swabs were tested as "blood swabs"
   COPAN cotton swabs, COPAN FLOQSwabs, Genotubes
- Blood samples from experimentally infected animals (ASFV/CSFV)
- Storage as dry swabs for at least for 24 h
- Immersion or fragment testing
- Nucleic acid extraction (manual/automated); one extraction for both diseases
- ASFV and CSFV specific PCR
- Comparison with EDTA blood samples of the same animal
- Additional tests with genotubes



## **Immersion/Viral Kit**





## Results



- ASFV and CSFV were reliably detected in blood swabs
- Immersion in lysis buffer and extraction from swab pieces worked well (the latter allows retesting)
- Manual and automated extraction methods could be used
- Genotubes had advantages in terms of cutting and storage

Animal	Inoculum	D.1	D.35
365	CSFV "CSF1047", gt 2.1	26.91	28.19
366	CSFV "CSF1047", gt 2.1	24.27	27.73
367	CSFV "CSF1047", gt 2.1	32.24	31.38
368	CSFV "CSF1047", gt 2.1	26.31	28.61
76	CSFV "CSF1045", gt 2.3	28.22	30.04

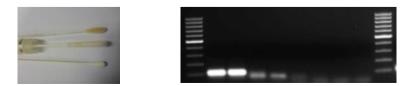
- Swabs could be used for passive swine fever surveillance in wild boar!
- Suitability for other diseases should be tested

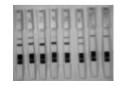




# **Pre-assay processing of samples**

- Easy to handle processing steps are crucial for the acceptance of downstream applications
- Use of techniques that can be used directly on the sample matrix (e.g. direct PCRs for blood, tissues, swabs)
- Safe immobilization and storage of diagnostic analytes (e.g. forensic swabs, FTA cards)
- Combination of methods to rational work-flows
- Evaluation of these techniques using well characterized reference samples
- RAPIDIA achievements: Workflows for swab sampling with subsequent direct PCR and detection of PCR products on NALF, optimization of deep-sequencing from FTA cards and filter paper

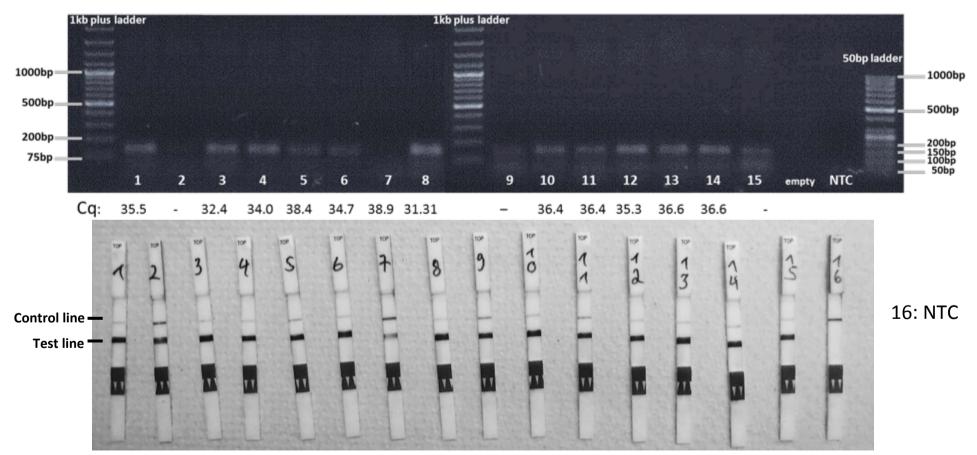






# **Direct PCR & NALF detection**

ASFV from Genotubes after 11 months of storage



## Virtual Database for references and sample materials



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		Porcine	Other	CSPV	
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			- UNDESFORSCHUNGSINSTITUT (FUI) (GERMANY)		
		Species of origen	Sample type	Pathology	
		Porcine	Other	CSPV	
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WP4: Antibody detecttion in the field

WP5: Pathogen detection in the field



# **Pen-site Applications**

- Test technologies such as lateral flow devices (LFDs) or certain genome amplification techniques can be transferred to the point of care
- Support decision-making "data-based suspicion"
- Investigation of disease syndromes with multiplex approaches
- Antibody detection using lateral flow devices
- Pathogen detection through the use of portable PCR, isothermal amplification technology (e.g. LAMP), and adpated LFD technology
- RAPIDIA achievements: e.g. ENIGMA FL and AMPLite validation, duplex antibody LFDs (ASFV/CSFV; AHSV/EIAV)







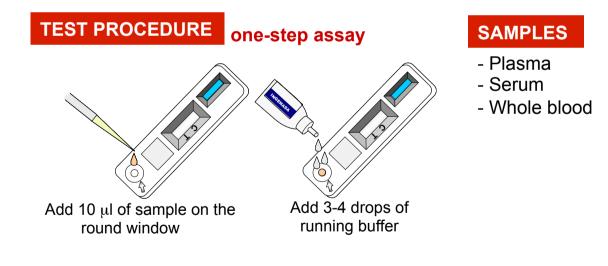




# **Lateral Flow Devices**

### Detection of antibodies directly in the field

### **INGENASA: CSFV/ASFV antibody detection**





### → good sensitivity and specificity

Molecular detection methods for FMDV in the field portable qPCR-device



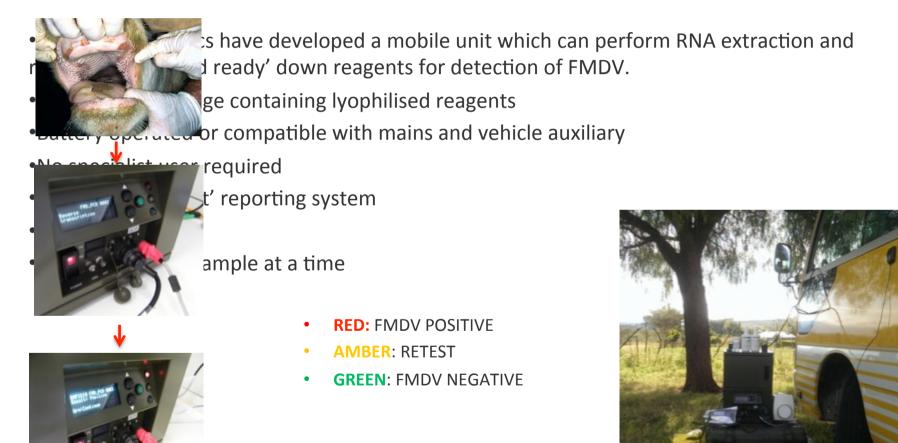


(Source: ENIGMA, UK)

www.pirbright.ac.uk

## Molecular detection methods for FMDV in the field

### Portable rRT-PCR platform (Enigma Field Laboratory - FL)



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## Validation of Enigma FL for the detection of FMDV:



- using **spiked epithelium** dilution series
- using archival field samples
- field validation in Kenya (FAO) and Tanzania (SUA/UoG)
- Results compared to gold-standard real-time RT-PCR (where possible)











Food and Agriculture Organization of the United Nations

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# **RT-LAMP-LFD Closed system (isothermal amplification and direct detection!)**

# To develop and perform initial validation of closed RT-LAMP device (AMPlite) for detection of FMDV at the pen-side

### What is the AMPlite?

- Closed system to minimise technical input required for assay and to minimise crosscontamination.
- Inexpensive heating block with disposable consumables.



- FMDV RT-LAMP assay was modified to incorporate a isothermal mastermix produced by Optigene.
- Performance of assay and device was compared against rRT-PCR and real time RT-LAMP/ RT-LAMP-LFD.



WP6: Antibodies detection in small field labs

WP7: Pathogen detection in small field lab



# Simple laboratory assays



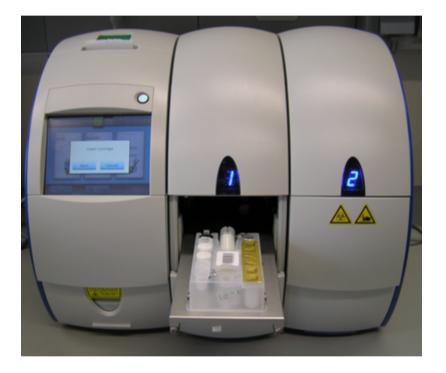
- Front-line laboratories are often ideally located but may lack infrastructure
- Manageable tools are needed that work under limited conditions
- Antibody detection through multiplex "comb" LFD devices, Quick ELISA formats, and low density microarray technology
- Pathogen detection e.g. through the use of isothermal amplification methods, multiplex antigen ELISA, DNA Chip systems, and AlphaLISA
- Multiplexing allows simultaneous detection of disease or species related clusters
- RAPIDIA achievements: Validation of ENIGMA ML assays (FMDV, ASFV/CSFV), design of microarrays for antibody detection, LAMP, RPA





Cartridge based ENIGMA® ML (MiniLab)

•automated nucleic acid extraction
•subsequent real-time PCR and printout of the results ('POSITIVE/NEGATIVE')
•easy-to-use and operators do not require specialist training
•freeze-dried assays: no cooling of the cartridge required and implementation in arid or tropical areas is feasible
•Can process six samples



## ENIGMA® ML



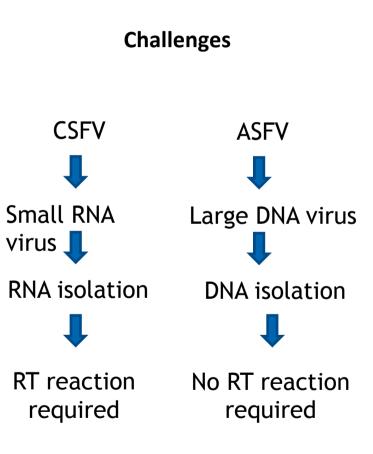
### Why multiplex ASFV and CSFV ?

### **Clinical symptoms**

### Similar for both diseases:

e.g. vomiting, diarrhoea (sometimes bloody), reddening or darkening of the skin (ears and snout), gummed-up eyes, laboured breathing and coughing, weakness and unwillingness to stand







### Validation of Enigma<sup>®</sup> ML for the detection of ASFV



10 fold blood dilution series from strains of genotype I, II and X  $\,$ 

Genotype I0	Enigma Printout	PCR manual extraction	Genotype II	Enigma Printout	PCR manual extraction	Genotype X	Enigma Printout	PCR manual extraction
Sardinia 1	pos	25.8	Lithuania 1	pos	22.2	Kenya 1	pos	25.5
Sardinia 2	pos	28.5	Lithuania 2	pos	25.4	Kenya 2	pos	28.7
Sardinia 3	pos	31.8	Lithuania 3	pos	29.3	Kenya 3	pos	31.4
Sardinia 4	pos	34.9	Lithuania 4	pos	31.9	Kenya 4	pos	35.0
Sardinia 5	neg	40.4	Lithuania 5	pos	36.5	Kenya 5	neg	38.3
Sardinia 6	neg	neg	Lithuania 6	neg	40.6	Kenya 6	neg	40.3
Sardinia 7	neg	neg	Lithuania 7	neg	neg	Kenya 7	neg	neg
Sardinia 8	neg	neg	Lithuania 8	neg	neg	Kenya 8	neg	neg
Sardinia 9	neg	neg	Lithuania 9	neg	neg	Kenya 9	neg	neg
Sardinia 10	neg	neg	Lithuania 10	neg	neg	Kenya 10	neg	neg
Sardinia neg	neg	neg	Lithuania neg	neg	neg	Kenya neg	neg	neg

 $\rightarrow$  detection of positive samples with cq values up to approximately 36-37; no false positives

 $\rightarrow$  diseased animals would be detected during acute infection: fit-for-purpose



# WP8: Confirmation and reference techniques



## **Reference techniques and Standardization**

- Confirmatory tests and special test applications
  - Molecular epidemiology
  - DIVA strategies
  - Pathotyping
  - Adaptation of techniques
- Next generation sequencing
- Luminex approaches
- Assessment of performance characteristics and validation
- Evaluation of new tests under defined laboratory and field conditions
- Commercialization  $\rightarrow$  ready-to-use products
- RAPIDIA achievements: Optimized NGS workflows for different settings, evaluation of new test systems towards commercialization



→ enormous sequencing capacity

**Next-generation sequencing** 

- $\rightarrow$  analysis of a whole range of sequence populations
- → Next-generation sequencing (NGS) allows metagenome analysis









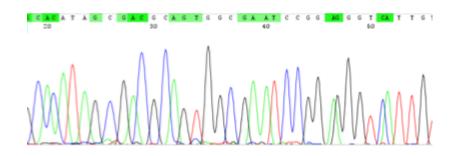


## NGS: general information

Advantages of NGS compared to conventional Sanger sequencing

Sanger sequencing:

- Sequence knowledge needed (PCR based)
- Sequencing of short genome fragments (~1000bp)
- Only one consensus sequence is generated



NGS:

- No sequence knowledge needed
- Sequencing of full genomes by assembly of overlapping sequences (50bp-~20kb)
- **Deep sequencing** can be conducted to identify variants

973 1023 1073 1123	Consensus	465 475 SAGCTCAGCA TGTCCA TA CCA
	Read 1 – Read 2 –	CATOTOCATACCA CATOTOCATACCA CATOTOCATACCA CATOTOCATACCA
		ACCTCA CATCTCCATACCA ACCTCA COTCCATACCA ACCTCA COTCCATACCA ACCTCACCATCTCCATACCA ATCTCCATACCA
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## **Examples**

- Detection of Schmallenberg virus and further classification of SBV within the Simbu serogroup
- Detection of the coexistence of three distinct genome variants within highly virulent BVDV-2 isolates
- In detail molecular evolutionary studies of CSFV type 2 sequences from outbreaks in wild boar from France
- Full-genome sequencing identified a tandem repeat insertion in Russian ASFV isolates

- Detection of unexpected novel diseases by NGS & metagenomics
- Detection of viral variants responsible for increased virulence



Suitability of NGS to obtain deeper molecular epidemiological knowledge

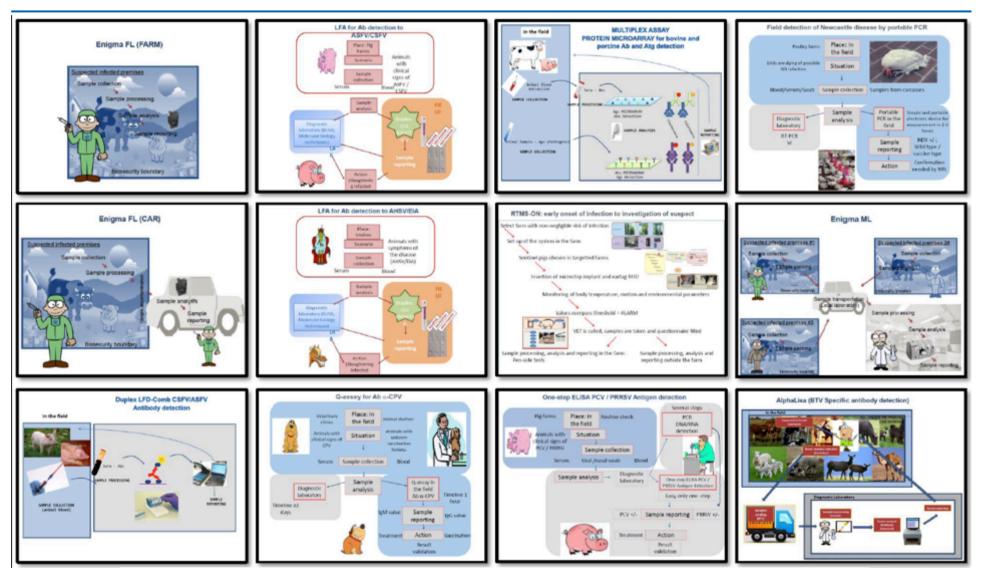


# WP9: Standardization

WP10: Dissemination of results

## **Diagnostic storyboards**





Creating simple storyboards permits visual narration and understanding of scenario flow (story components).

# **PART 4: Results ASFV FNG**

### • Sardinia (LFD Prionics):

- 57 wild boar samples (37 positive; 20 negative)
  - 1 false neg and 1 false pos in comparison with ELISA and immunoblot (= 1,7%)
  - 56/57 samples correctly identified (96,5%)
  - Se = 36/37 = 97,3%
  - Sp = 19/20 = 95,0%
- 41 pig samples (20 positive; 21 negative)
  - 1 false pos in comparison with immunoblot (= 2,4%)
  - 40/41 samples correctly identified (97,6%)
  - Se = 20/20 = 100%
  - Sp = 20/21 = 95,2%

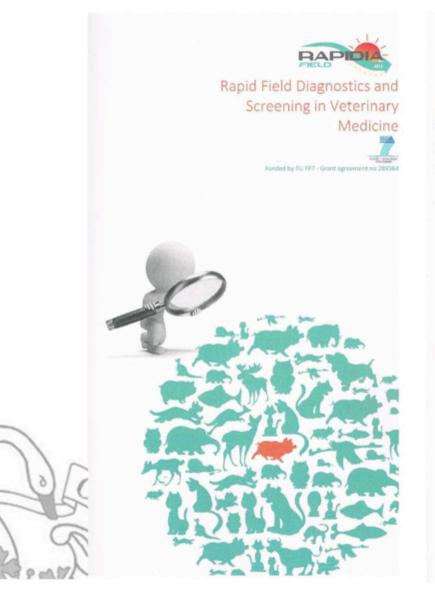
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PUBLICATIONS	
ARTICLES .	
🔁 Early detection of infection in pigs through an online monitoring system	21 peer-
🔁 Molecular Approaches to Recognize Relevant and Emerging Infectious Diseases in Animals	
🔁 Tandem Repeat Insertion in African Swine Fever Virus, Russia, 2012	reviewed
🔁 Development of a Microsphere-based Immunoassay for Serological Detection of African Horse Sickness Virus and Comparison with C	Other Diagnostic Technic publications
Development of a loop-mediated isothermal amplification assaycombined with a lateral flow dipstick for rapid and simple detection in the field	
🔁 Recovery of Viral RNA and Infectious Foot-and-Mouth Disease Virus from Positive Lateral-Flow Devices	COMMUNICATIONS
Preliminary Validation of Direct Detection of Foot-And-Mouth Disease Virus within Clinical Samples Using Reverse Transcription Lo Amplification Coupled with a Simple Lateral Flow Device for Detection	2014
🗏 Rovac is the possible ancestor of the Russian Lapinized vaccinesLK-VNIVVIM and CS strains but not the Chinese strain (C-strain)vacci feverWeiguang	<ol> <li>Powler VL et al. (2014). Keatising the potential of pensioe testing for foot-and-mouth disease. Invited guest talk, Kingston University. November 19th 2014.</li> <li>Fowler VL et al. (2014). Development and evaluation of multiplex reverse transcription loop mediated isothermal amplification assays combined with lateral-</li> </ol>
🔁 Alternative sampling strategies for passive classical and African swine fever surveillance in wild boar	flow visualisation for the discrimination of FMD from other vesicular diseases. Open Session of the European Commission for the Control of Foot-and-Mouth Disease Vibere science and policy meet: FMD RISK MANAGEMENT in a world of changing disease landscapes 29-31 October 2014, Cavtat, Croatia.
2 Development and validation of rapid magnetic particle based extraction protocols	<ol> <li>Martínez Avilés M., Fernández Carrión E., Bernal-Orozco I., Rivera B., Mazariegos M., Sánchez-Vizcaino J.M. (2014). Early detection of transboundary animal diseases with a real-time monitoring system online. Bith Annual Epizone Meeting. Copenhaguen, Denmark.</li> </ol>
🖻 Mixed triple: allied viruses in unique recent isolates of highly virulent type 2 bovine viral diarrhea virus detected by deep sequenci	
a Assessment of Preparation of Samples Under the Field Conditions and a Portable Real-Time RT-PCR Assay for the Rapid On-Site De Virus	<ol> <li>Petrov A, Schotte U, Pfetschmann J, Dräger C, Beer M, Anheyer-Behmenburg H, Goller KV, Biorne S (2014). Alternative Beprobungsstrategien: Molekularer</li> <li>Nachweis Afrikanischer und Klassischer Schweinepest bei Fallwild -Eignung von Tupferproben. 33rd AVID-Tagung, Kloster Banz, Bad Staffeistein, Germany, September.</li> <li>Petrov A (2014). Alternative sampling strategies in wild boar: The use of swabs for passive classical and African swine fever surveillance in wild boar.</li> </ol>
amplification assay combined with a lateral flow dipstick for rapid and simple detect virus in the field	
🔁 False-Positive Results in Metagenomic Virus Discovery: A Strong Case for Follow-Up Diagnosis	<ol> <li>Goller KY (2013). Possible methods for disease identification in the KAZA region developed in the RAPIDIA field project. 2nd KAZA workshop, Johannesburg, South Africa, December.</li> </ol>
🐔 silinad Volalas sillad idawaa la calaca leelstaa af waxaa blabliceleedaat tewa 9 bacdaa chest disedaas idaw 190900 95 datastad bii daa	<ol> <li>King D. P., Armson B., Moulet V., Madi M. and Fowler V. (2014). Simple field tools for the diagnosis of livestock diseases: is this an achievable goal? 9th Conference of Rapid Methods Europe, Noordwijkerhout, The Netherlands, April.</li> </ol>
	<ol> <li>King D.P. (2014). New Tools to Detect and Monitor the Spread of Viral Diseases of Livestock" i European Animal and Plant Symposium, Amsterdam. 25 February.</li> </ol>
	<ol> <li>Sánchez-Vizcaíno, JM (2014). Conferencia internacional PESTE PORCINA AFRICANA. SZIE Faculty of Veterinary Science, Budapest, 5 March.</li> <li>Giménez-Lirola, L., Mur, L., A.Mogler, M., Lizano, S., K.Gppdell, C., Sánchez-Vizcaíno, JM., DL (Hark) Harris, Zimmeman, J. (2014). RNA particles: A novel</li> </ol>
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	Barcelona. 29-31 January. 15. Belak, S.: Recent trends and developments in molecular diagnostic virology and infection biology. Seminar and Scientific Meeting, Animal Health Laboratory
	Investigation and Diagnostic Centres, Upper Hutt New Zealand, 25 February 2014. Invited speaker. 16. Beläk, S.: Recent trends and developments in molecular diagnostic virology and infection biology. Report from the OIE Collaborating Centre for the
60	Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine. Seminar and Scientific Meeting, Australian Animal Health Laboratory, Geelong, Australia, 7 March 2014. Invited speaker.
	<ol> <li>Belák, S.: New tendencies and recent developments in molecular diagnostic virology and infection biology. Recent results of the OIE Collaborating Centre for the Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine. Seminar and Scientific Meeting, Australian Animal Health Laboratory, CAFHS,</li> </ol>
communications in	no. Australia, 10 March 2014. Invited speaker approaches, achievements and developments in the molecular diagnosis of infectious diseases. Seminar and discussions at EICMP, Dubai, United
23 different	15 March 2014. Invited speaker. 2. New Viruses In veterinary medicine, detected by metagenomic approaches. Seminar and Scientific Meeting, Australian Animal Health Laboratory, and a local science of the second
	<ol> <li>Geelong, Australia, 11 March 2014. Invited speaker.</li> <li>Petror A, Schotte U, Pietschmann J, Dräger C, Beer M, Anheyer-Behmenburg H, Goller KV, Blome S (2014). Alternative sampling strategies for passive classical distribution of the second strategies of the second strategies of the second strategies. Second strategies for passive classical distribution of the second strategies of the second strategies of the second strategies. Second strategies for passive classical distribution of the second strategies for passive classical distribution.</li> </ol>
countries	and African swine fever surveillance in wild boar. 8th Annual EVIZONE meeting, Copenhagen, Denmark, September. (Poster) 21. Sastre P., Pérez T., Tapia I., Sánchez-Matamoros A., Sánchez-Vizcaíno J.M., Rueda P., Sanz A. (2014). New diagnostic tools for African Horse Sickness and Equine Infectious Anemia viruses control. 8th Annual Epizone Meeting, Copenhaguen, Denmark. (Poster)
12/11/2000	2013



# **Dissemination** leaflet



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Sampling	7
Field/Small lab tests	13
Immunochromatographic test or lateral flow assay (LFA)	
LFD-Comb	
One-step ELISA for Antigen detection	
Rapid assay for IgG and IgM detection in immunotubes.	
Magnetic immunoassay (MIA)	
Recombinase Polymerase Amplification (RPA)	
Fully automated PCR designed for non-expert users in non-laboratory settings	
Confirmatory tests	

# RAPIDIA WORKSHOP, Tuesday, 20/05/14, MADRID





#### Around 40 participants

WORKSHOP MADRID, SPAIN MAY 20TH 2014

DESTITUTION	NAME
or	Barbara Prelecters (Executive Director, Inter- national Pederation for Animal Health (FAH))
CIE reference lab for AHSV (Spain)	Monthemat Agilero Gancia (Tecnical Director, Lateratore de Canical Anima, MAGRASIA) Manual Durán Peres Ruben Villalto Martinez
OIE reference teb for AHEV (UR)	Javler Caeblo-Obvares (DE designated expert on African horse sickness)
OIE reference lab for ASPV (Spain)	Represented by partner of the consortium (UCM-VIBAVET)
OEIEUFIO World Reference Laboratory for FMDV (UR)	Represented by partner of the consortium (PK)
ORDEU Raferance Laboratory for SVD-(UK)	Represented by partner of the consortium (PK)
CVO Hungary	Aboryl Tamás (Director of the Veterinary Diag- nosts Directorale (VDD) of the National Food Chain Safety Office (NFCSD))
CVO Inland	Bonal Sammin-Director of the Department of Agriculture Food and Marine Velennary Laborato- ries)
CVO Spain	Luis Romero Gonzalez (Chief of Epidemiology Department, MAGRAMA)
CVO UK	Simon Hall /veterinary Director of our Animal Health Veterinary Laboratories Agency).
EU producers (COPA- COGECA)	Nigel Angel Higuera (ASAJA, ES), Vice-chair of the Copa-Cogeca Animal Health and Welfare Working Party
European refer- ence lab for ASFV	Marlea Arlae (Tecnical Director, CIGA-NIA)
Plataform Vet-I	Pablo Hervile Calle (Teorical Decretary Vel+)
EWVD	Belen Barreiro (Vicepresident)
Members of Advis	ary Beard
Contail University	Alfonso Torres (Professor & Associate Dean for Public Policy College of Veterinary Medicine)
Iowa State Uni- versity	Jeffrey Zimmerman (Professor of Velerinary diagnostic laboratory)
Scientific Officer	Luis Vinas-Alegre



"Rapid field diagnostics and screening in veterinary medicine" Funded by EU FP7, Grant agreement no 289364



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00-0815	thecome and introductory remarks	Prof. Pedro Lorenzo Gean di Velennary Paculty Prof. J.N. Ganchez-Vizcarlo VISAVET-UCM
15-09.30	RAPIDIA-PIELD AT A GLANCE	Dr. Paloma Rueda INGENASA
30-09.45	Scientific introduction	Prof. Martin Beer PLI
46-12.05	WP2. Real— time monitoring livestock on line	Prof. J.W. Sanchez-Vizcaino VISAVET-UCM
05-10.25	WPS. Collection and prep- aration of samples	Dr. Veronica Powier PdR
25-10.45	WP4, Antibody detector in the field	Or. Patrica Saltre INGENAGA
	COFFEE BREAK	
10-1128	WP5. Pathogen defection in the field	Prof. Sandor Belak SVA
38-11.58	WP5. Pathogen delection in the field WP4. Antibody delection in small labs	
	delector in the field	State Rither
39-11.98	Selector in the field WPL Antibody Selector in small ups	SVA Dr. Alex Riber PRIONICS Dr. Sandra Biome
38-11.58 58-12.15	Selector in the field WP4. Antibody delector in small labs WP7. Pathogen delector in small labs WP4. Confirmation and	SVA Dr. Alex Raber PRISONOS Dr. Sandra Biome Ful Dr. Katja-Galler
38-11 88 59-12 18 16-12 38	Selection in the field IBPE, Antipody defection in small labs IBPF, Conformation and inference techniques IBPS, Conformation and IBPS, Conformation and IBPS, Conformation and IBPS, Conformation (IBP)	SVA Dr. Axe Riber PRONOCS Dr. Sandra Biome FU Dr. Kalja Goller FU Dr. Fask Koenen

1.0	PROGRAMME
Vine i	Packages Overview

21.00		LCARAVEA RESTAURAN
16.15-17.15	FINAL DISCUSSION	
	COFFE BREAK	
15.30-16.00	Guest presentation	Dr. Barbara Freischem Old
15.00-15.30	Scientific Officer Presentation	Dr. Lois Vivas-Alegre

#### RAPIDIA-FIELD Consortium

1. Inmunciogía y Genética Aplicada SA (INGENASA, Spain)

- 2. Friedrich-Loeffler-Institut (FLI, Germany) 3. PRIONICS (Switzerland)
- 4. PROPHYL (Hungary)
- 5. Instituto Nacional de Técnica Aeroespacial, Centro de Astrobiologia (INTA-CSIC, Spain)
- 6. The Pirtright Institute (PIR, United Kingdom)
- 7. Agence Nationale de Sécurité Sanitaire de l'Alimentation de Environnement et du Traval (ANSES, France)
- 8. Universidad Complutence (VISAVET-UCM, Spain)
- 9. CODA-CERVA (Belgium)
- 10. The National Veterinary Institute (SVA, Sweden)
- 11. IDEXX SWITZERLAND AG (Switzerland)
- 12. ENIGMA DIAGNOSTICS LIMITED (United Kingdom)



## INGENASA

## Thanks for your attention!



















