ASF Nanogold Biosensor Test Kit:
An Alternative Decentralized ASF Detection Assay

Clarissa Yvonne J. Domingo, Rizalee S. Pilare, Twinkle G. Barangan, Lilet C. Cruz

Global African Swine Fever Research Alliance (GARA) Gap Analysis December 5 - 7, 2023 in Manila, Philippines

Have you ever witnessed the heart-wrenching sight of a wailing mother when the African Swine Fever (ASF) virus strikes her pigs?
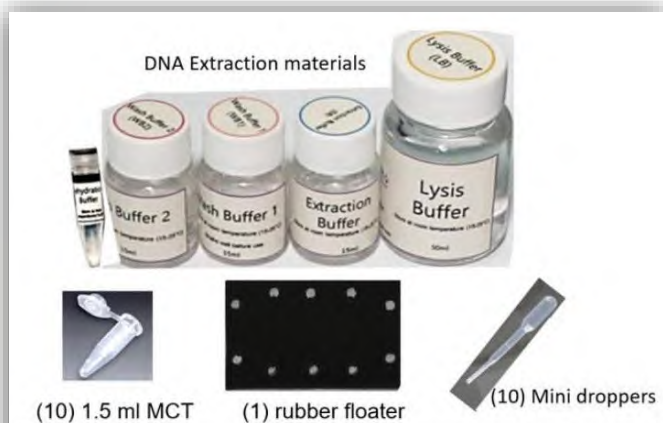
COMPONENTS

Uses simple machines

microcentrifuge

dry bath with heat block

DNA Extraction materials

Lysis Buffer (LB)
Buffer 2
Wash Buffer 1
Extraction Buffer
Lysis Buffer

(10) 1.5 ml MCT   (1) rubber floater   (10) Mini droppers

QTY	VOL	REF
10		MCT Tubes
10		Mini Droppers
1		Rubber Floater
1	7 mL	Lysis Buffer (LB) store at room temp. inside the box
1	8 mL	Extraction Buffer (EB) store at room temp. inside the box
1	7 mL	Wash Buffer 1 (WB1) store at room temp. inside the box
1	5 mL	Wash Buffer 2 (WB2) store at room temp. inside the box
2	0.5 mL	Rehydration Buffer (RB) store at room temp. inside the box

COLORIMETRY

Blood and Raw/Processed Pork Samples	Fecal/Oral/Envi Swabs and Water Samples
(STRONG) POSITIVE (WEAK)	(STRONG) POSITIVE (WEAK)
NEGATIVE	NEGATIVE

Comparative Analysis of ASF Nanogold Biosensor Test Kit

| Parameters	ASF Nanogold Biosensor	Conventional/Real-Time PCR	ELISA
Time required (hrs) from sample prep to result	2 hours (DNA extraction until reading of results)	6 hours (DNA extraction until reading of results)	5 hours (from receipt of samples to result)
What is detected	Viral genetic material	Viral genetic material	Antibodies
Applied to a wide variety of samples	Yes (versatile)	No (Selective)	No (Serum only)
Sensitivity	90 to 100%; best used for early detection (infected but asymptomatic pigs – incubating stage)	Seroconversion gives false negative in RT PCR; for confirmatory use only on acute cases (with symptoms)	Reliability affected by inhibitors or contaminants
Equipment needed	Dry bath & microcentrifuge	RT PCR/gradient thermal cycler, gel electrophoresis, gel documentation	Incubator, ELISA reader & washer
Cost of Equipment	80K	3M	800K
Cost/test	Php 400/test	Php 1000 to 3,200/test	Php 500/test

3X faster than PCR

2-8X more affordable than PCR

for early ASF detection; more sensitive than Real-Time PCR

VERSATILE ON DIFFERENT SAMPLES

Envi Surface Swabs
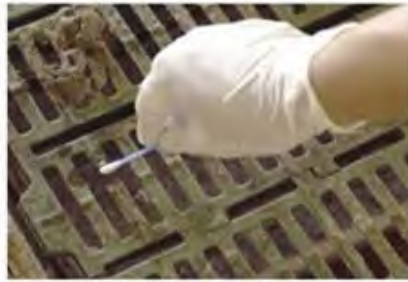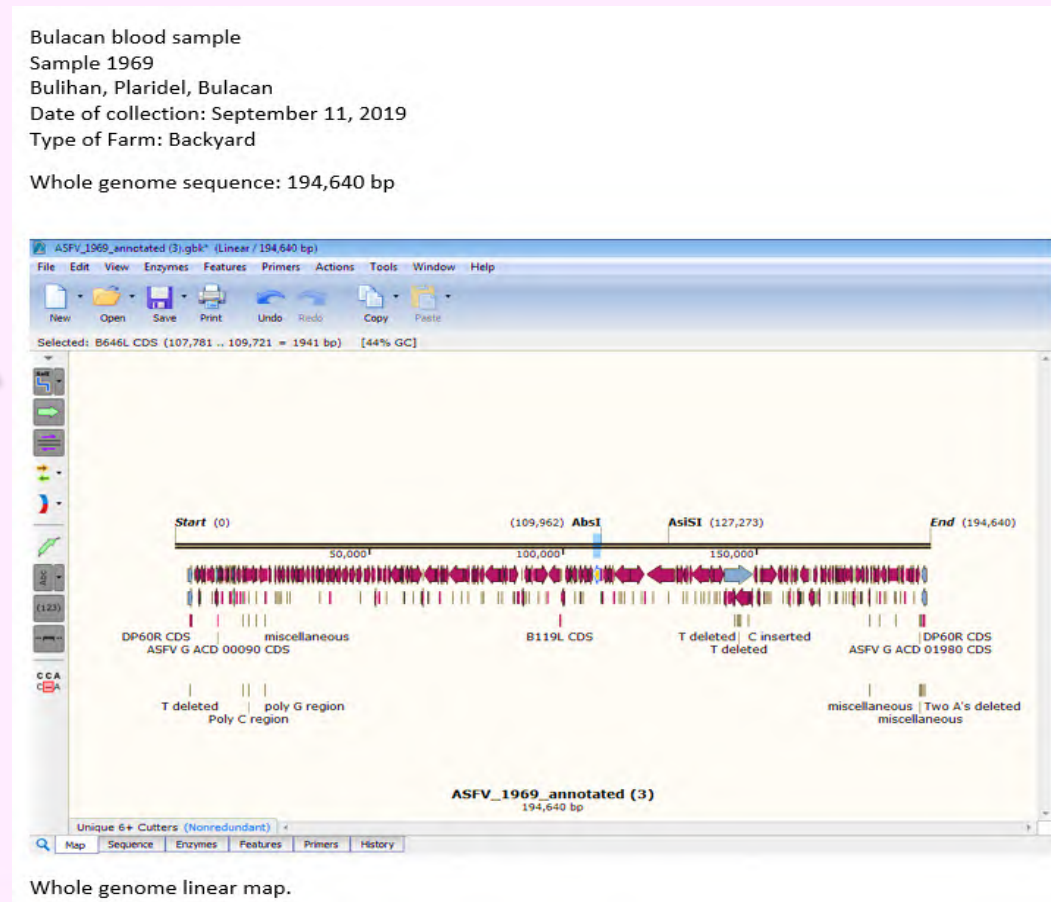
Oral & Rectal Swabs

Water

Blood from ear vein

Hog feeds

Raw and processed meat

5 gm of meat placed in clean ziplock & transported in cold storage

Meat homogenate Ready for DNA extraction

Design of LAMP primers (3 pairs) was based on the whole genome sequence of p72 (B646L) acquired from an ASF-infected blood sample in Bulacan in 2019 as reference.

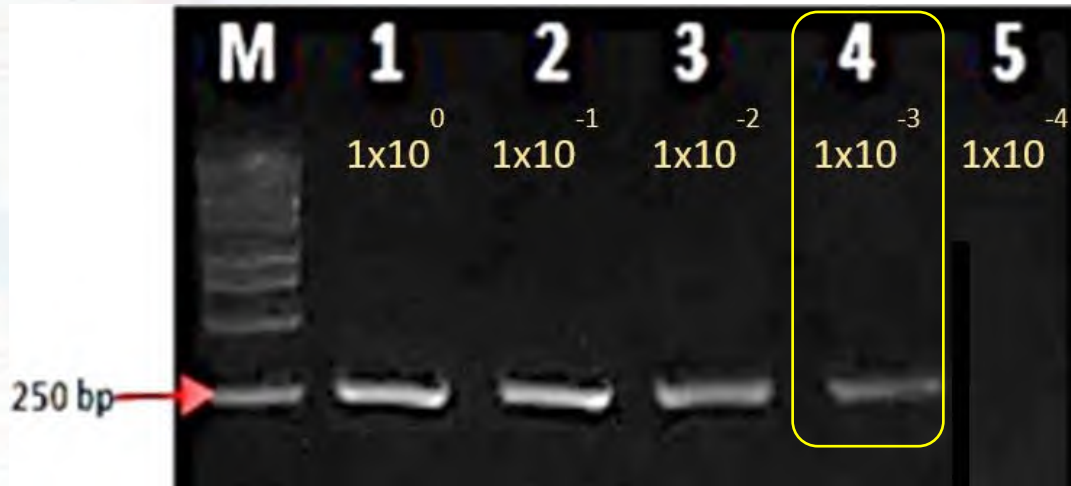Whole genome linear map.

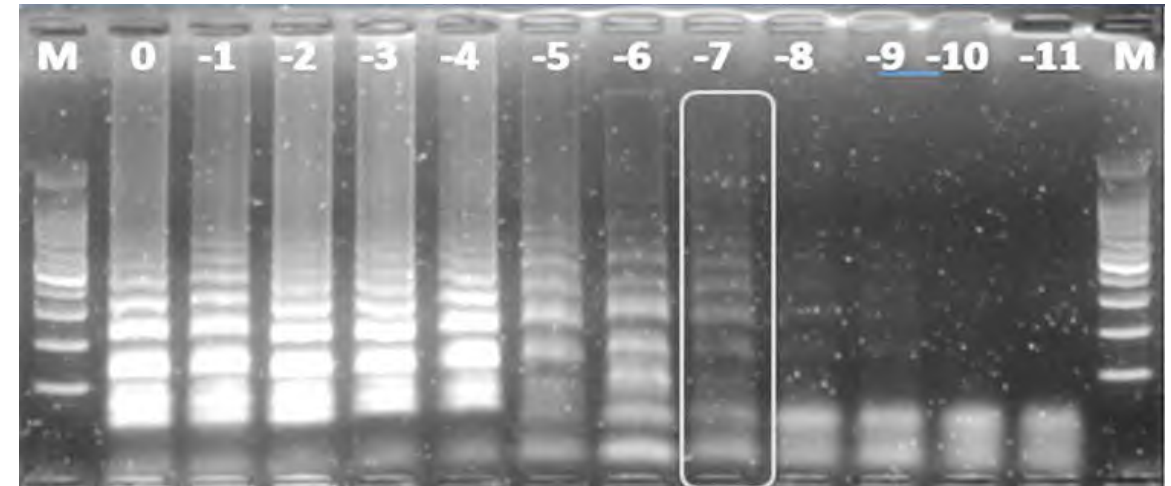For conventional PCR, the outer F3/B3 LAMP primers were used.

ANALYTICAL SENSITIVITY BETWEEN LAMP and CONVENTIONAL PCR

CONVENTIONAL PCR USING OUTER F3/B3 LAMP PRIMERS

LAST LANE 10^{-3} WITH FAINT BAND

GEL IMAGE OF ELECTROPHORESIS RESULT OF LAMP SENSITIVITY ON BLOOD SAMPLES
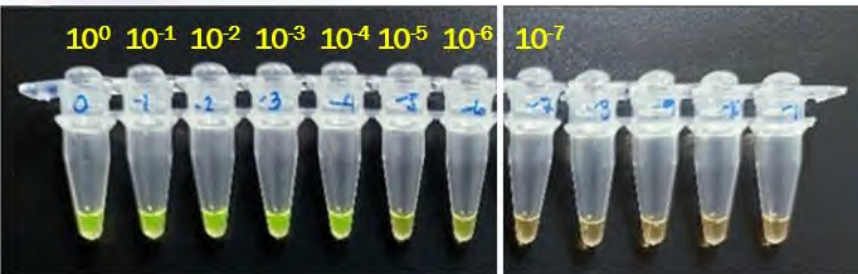

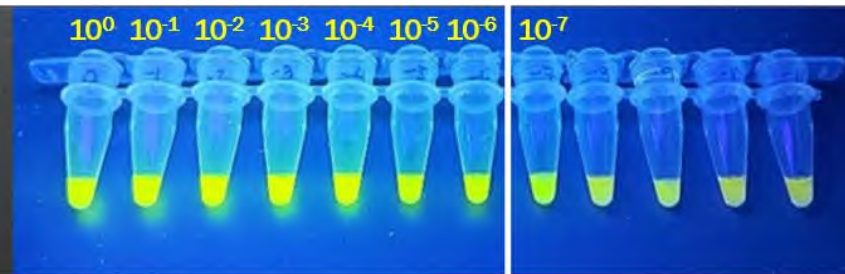
LAST LANE 10^{-7} WITH MULTIPLE BANDS SEEN

LAMP amplification is more sensitive than conventional PCR despite using the outer F3/B3 LAMP primers as PCR markers implying the LAMP's enhanced sensitivity over PCR due to having 3 pairs of primers.
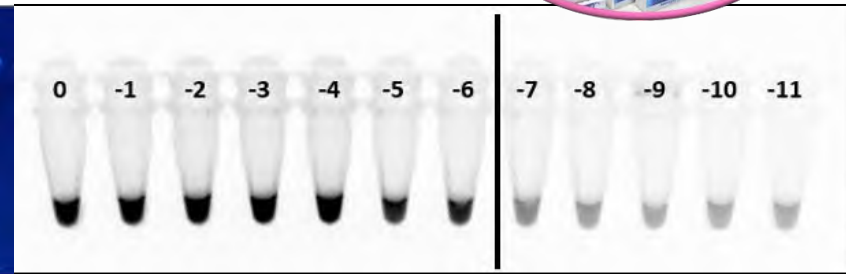
LAMP ANALYTICAL SENSITIVITY

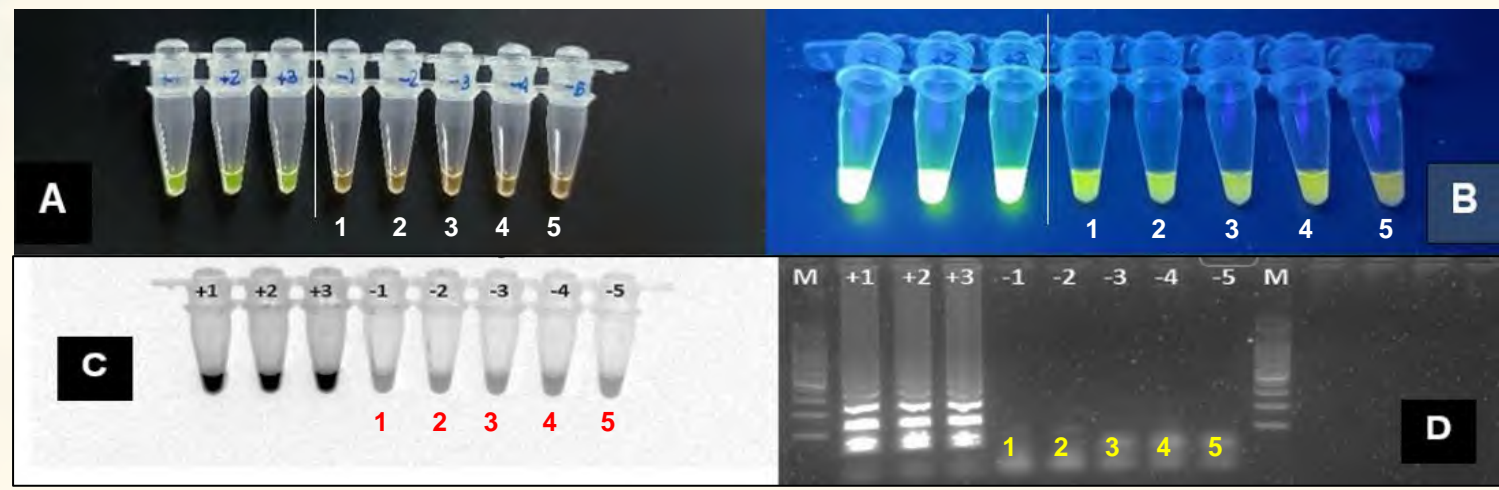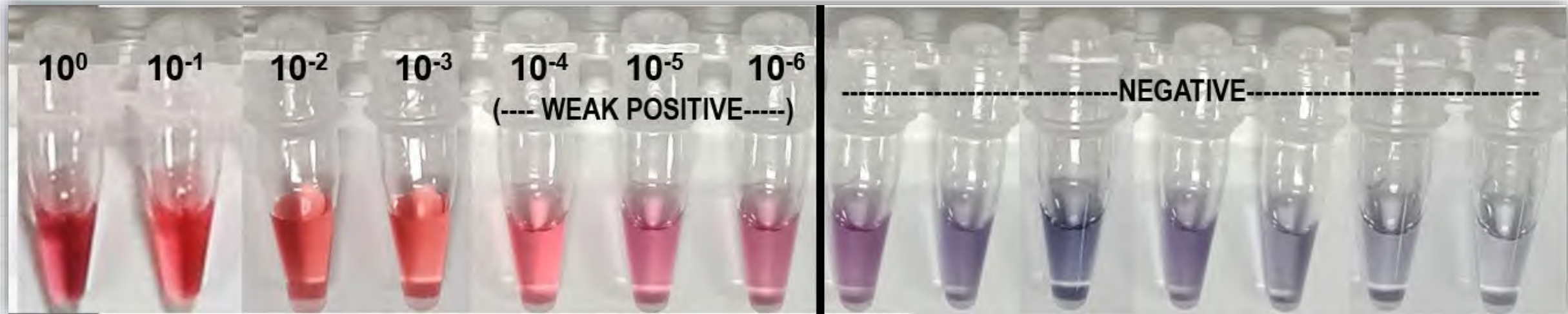Dye Test   Fluorescence Test   Turbidity Test

LAMP ANALYTICAL SPECIFICITY

A. Dye Test
B. Fluorescence Test
C. Turbidity Test
D. Gel Electrophoresis Test

1 - Classical Swine Fever virus
2 - PRRS virus
3 - PRV
4 - PEDV
5 - no DNA template (DDW)

ANALYTICAL SENSITIVITY OF LAMP-AuNP IN BLOOD & TISSUE SAMPLES

10^0 10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6}
(---- WEAK POSITIVE-----)

------------------------------NEGATIVE----------------------------------

ANALYTICAL SPECIFICITY OF LAMP-AuNP IN BLOOD & TISSUE SAMPLES

Blood **Tissue** **CSF** **PRRS** **IAV** **RV**
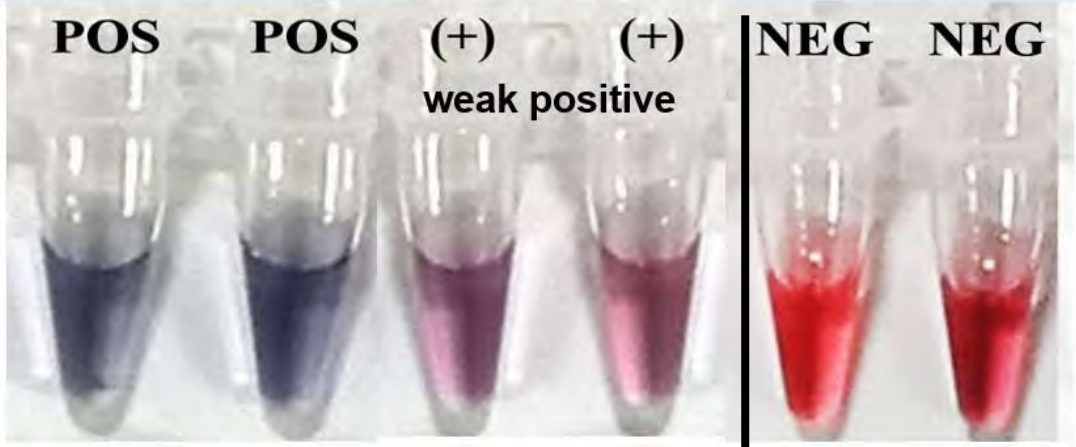**(+)** **(+)** **(-)** **(-)** **(-)** **(-)**
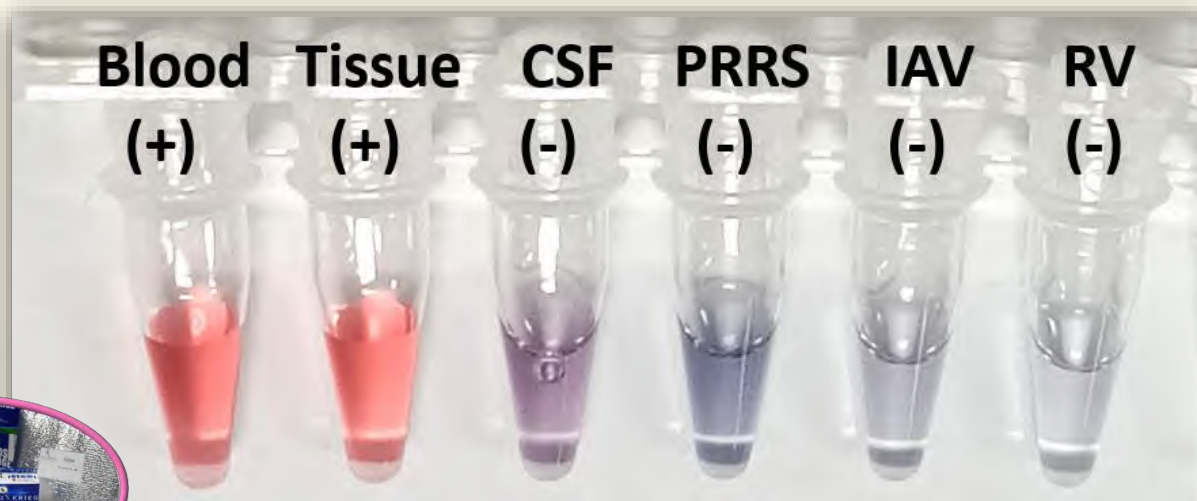
Blood- positive sample from Tarlac
Tissue- positive samples from Pangasinan
    CSF *(Classical Swine Fever)*- vaccine virus
    PRRS *(Porcine Reproductive and
        Respiratory Syndrome)*- vaccine virus
    IAV *(Swine Influenza Virus)*- positive field
        sample from 2018
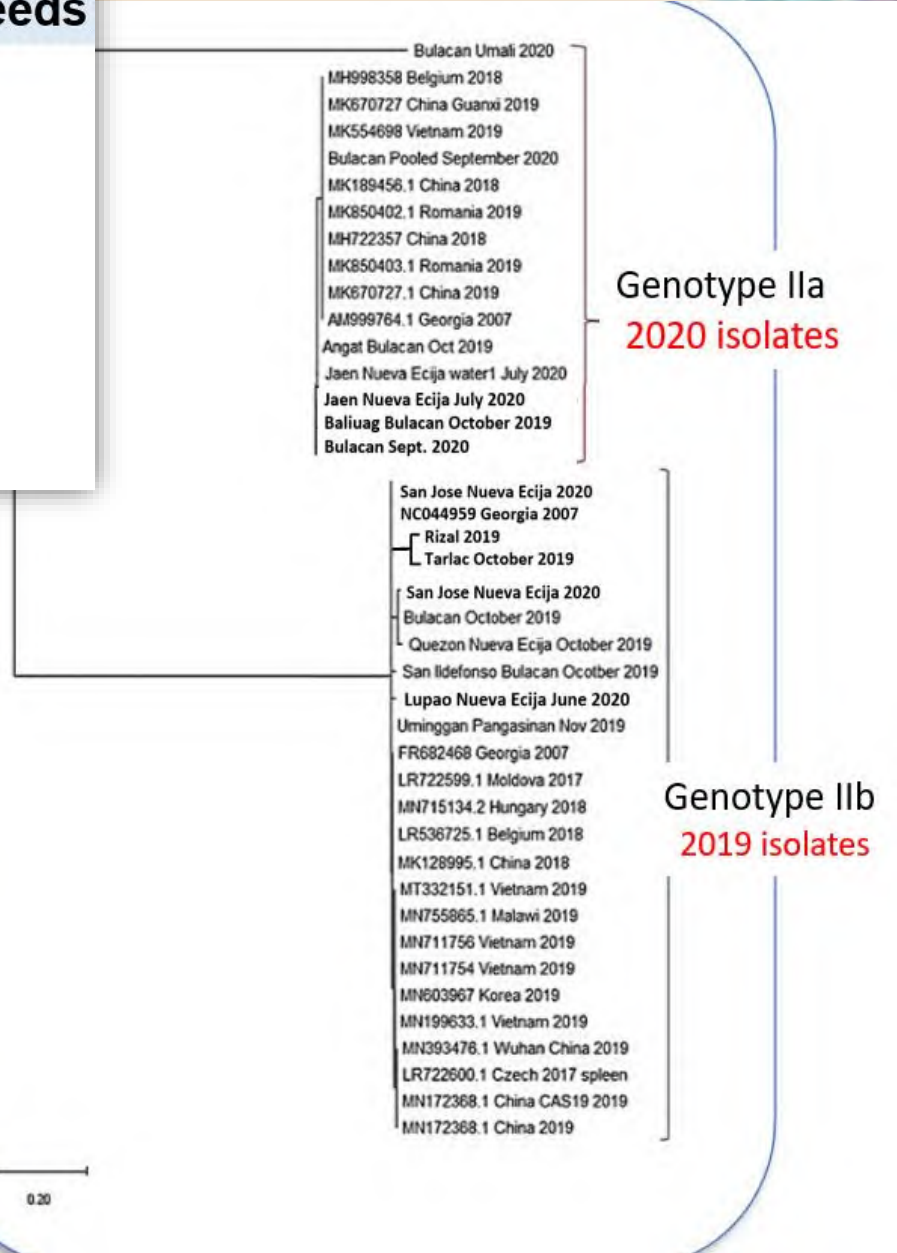   **RV** *(Rotavirus)* -plasmid

Environmental Swabs, Oral and Fecal Swabs, Water, Feeds

POS POS (+) (+) NEG NEG

weak positive

Phylogenetic and phylogeographic analysis of the detected ASFV isolates

1 field isolate – spleen, index case in Tanay, Rizal
10 field isolates – blood
4 field isolates – surface swabs
3 field isolates – water
1 field isolate – feces

Bulacan Umali 2020
MH998358 Belgium 2018
MK670727 China Guanxi 2019
MK554698 Vietnam 2019
Bulacan Pooled September 2020
MK189456.1 China 2018
MK850402.1 Romania 2019
MH722357 China 2018
MK850403.1 Romania 2019
MK670727.1 China 2019
AM999764.1 Georgia 2007
Angat Bulacan Oct 2019
Jaen Nueva Ecija water1 July 2020
Jaen Nueva Ecija July 2020
Baliuag Bulacan October 2019
Bulacan Sept. 2020

Genotype IIa
2020 isolates

San Jose Nueva Ecija 2020
NC044959 Georgia 2007
Rizal 2019
Tarlac October 2019
San Jose Nueva Ecija 2020
Bulacan October 2019
Quezon Nueva Ecija October 2019
San Ildefonso Bulacan Ocotber 2019
Lupao Nueva Ecija June 2020
Uminggan Pangasinan Nov 2019
FR682468 Georgia 2007
LR722599.1 Moldova 2017
MN715134.2 Hungary 2018
LR536725.1 Belgium 2018
MK128995.1 China 2018
MT332151.1 Vietnam 2019
MN755865.1 Malawi 2019
MN711756 Vietnam 2019
MN711754 Vietnam 2019
MN603967 Korea 2019
MN199633.1 Vietnam 2019
MN393476.1 Wuhan China 2019
LR722600.1 Czech 2017 spleen
MN172368.1 China CAS19 2019
MN172368.1 China 2019

Genotype IIb
2019 isolates

0.20

10 qPCR positive*
9 BADC PCR positive**

10 qPCR negative
5 BADC PCR negative

M T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12 | M T13 T14 T15 T16 T17 T18 T19 T20 T21

1 2   3 4 5   ddw

124bp

*qPCR adopted from ASF Taqman probe used in the qPCR protocol (King et al. 2003)
**BADC PCR primers adopted from whole p72 genome of 2019 Bulacan ASF isolate

Gene sequences of the positive amplified products using BADC PCR primers that are negative in qPCR
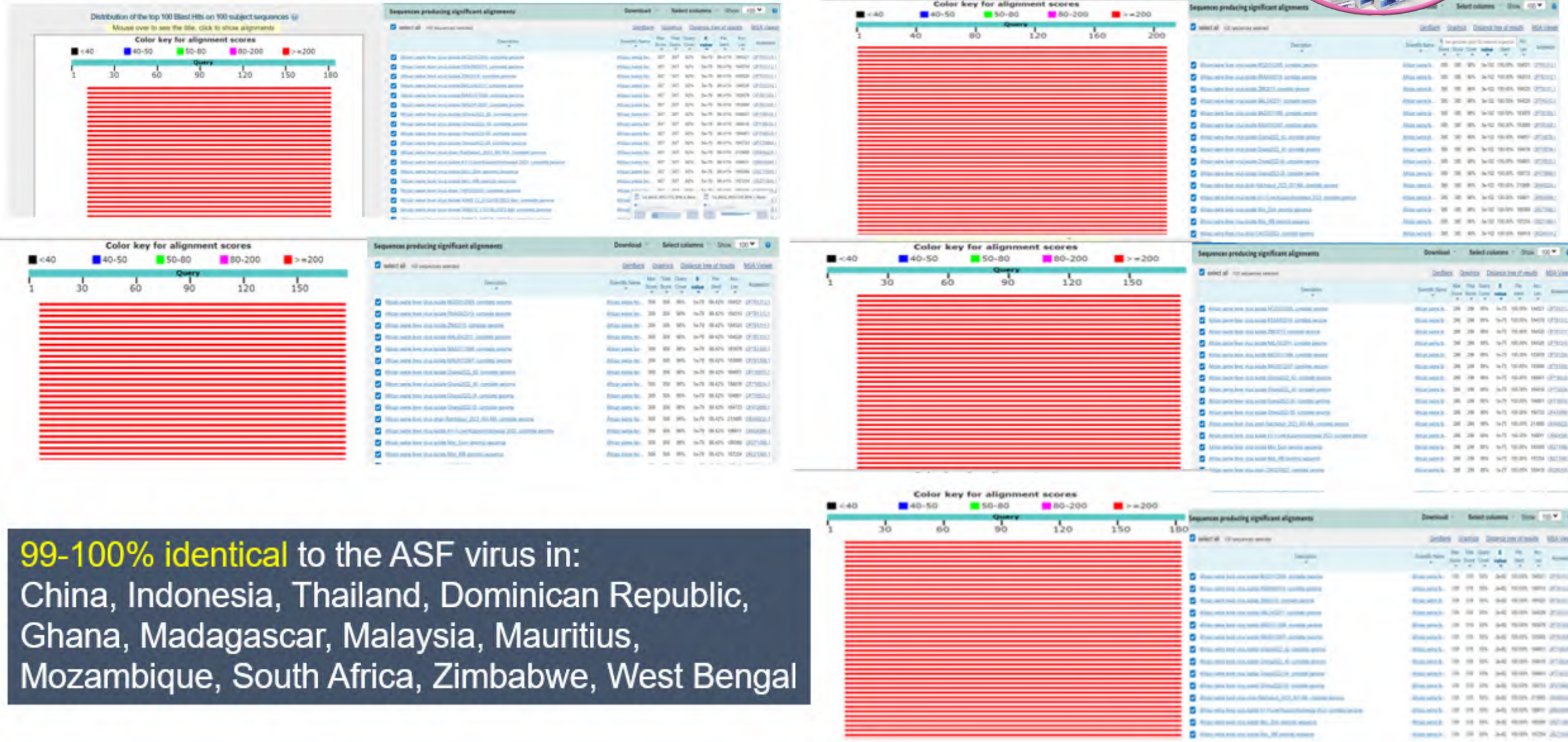
99-100% identical to the ASF virus in:
China, Indonesia, Thailand, Dominican Republic, Ghana, Madagascar, Malaysia, Mauritius, Mozambique, South Africa, Zimbabwe, West Bengal

Table 1. Diagnostic validity of ASF Nanogold Biosenensor with qPCR

Samples	Positi-vity Rate	% Sensi-tivity	% Specifi-city	% PPV	% NPV	Kappa Coefficient / Interpretation	% Accu-racy
Surface Swab (n=40)	47.5	100.0	76.2	94.58	100.0	0.75 (substantial)	87.5
Water (n=40)	50.0	90.0	85.0	96.14	67.17	0.75 (substantial)	87.5
Fresh Meat (n=40)	82.5	100.0	85.7	96.68	100.0	0.91 (almost perfect)	97.5
Processed Meat (n=40)	82.5	97.0	85.7	96.58	87.2	0.83 (almost perfect)	95.0
Whole Blood (n=67)	80.6	96.3	77.0	94.55	83.33	0.75 (substantial)	92.5

Table 2. Comparison of the positivity rate of different samples using the ASF Nanogold Biosensor and conventional PCR

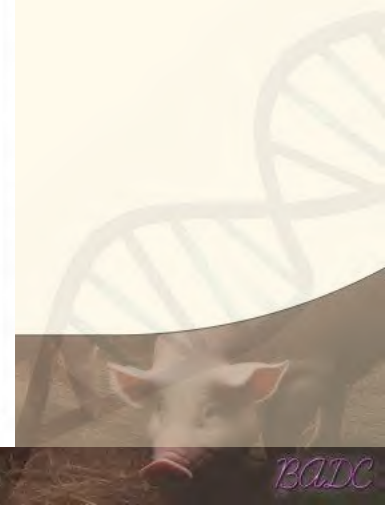| Sample Type (n=total samples)	ASFV Nanogold Biosensor		Conventional PCR	
	No. Positive Samples	Positivity Rate %	No. Positive Samples	Positivity Rate %
Blood (n=891)	605	67.9	357	40.0
Meat (n=881)	307	34.5	225	25.5
Oral Swab (n=32)	18	56.25	9	28.0
Fecal Swab (n=429)	181	42.2	82	19.11
Surface Swab (n=532)	156	29.3	61	11.5

Table 3. Comparison of the positivity rate of water samples using the ASF Nanogold Biosensor and conventional PCR

Test Assay (n=82 farms)	Positive	Positivity Rate %
ASFV Nanogold Biosensor	67	81.2
Conventional PCR	19	23.2

Table 4. Positivity rate of commercial feeds tested with ASF Nanogold Biosensor test kit

Time Sample Received (n=total # of samples)	Positive	Positivity Rate %
Early 2021 (n=15)	8	53.3
Early 2022 (n=11)	3	27.3
Total Samples (n=26)	11	42.3%

IPR APPLICATION FOR ASF TEST KIT

ASFV NANOGOLD BIOSENSOR TEST KIT – PATENT APPLICATION

INTELLECTUAL PROPERTY OFFICE OF THE PHILIPPINES
IP Center, 28 Upper McKinley Road, McKinley Hill Town Center,
Fort Bonifacio, Taguig City 1634 PHILIPPINES
T: +632-2386300 * F: +632-5539480 * www.ipophil.gov.ph

INTELLECTUAL PROPERTY
OFFICE OF THE PHILIPPINES

SERAPION, Jerry C
c/o CLARISSA YVONNE J. DOMINGO, Blitzkrieg
Animal Diagnostic Center, D. Delos Santos St.,
Población West, Science City of Muñoz, 3119,
Nueva Ecija

Application No. : 12023050074

Reference Code : 6DB02LH21H620237IP

Received Date : 21 February 2023

Applicant : Central Luzon State University

Title : METHOD AND TEST KIT FOR
DETECTING AFRICAN SWINE FEVER
VIRUS

This is to acknowledge receipt of your payment for the above-identified application
on 21 February 2023.

The application shall be assigned an Application Number upon payment of the
required fees. However, the application will not be considered as a bona fide
application if there is failure to pay the required fees in full upon filing of the
application and will be deemed as failed application pursuant to Rule 401.1 of the
Revised Implementing Rules and Regulations for Patents, Utility Models and
Industrial Designs of 2022

Endorsement
DA National Livestock Program

Emergency Use Authorization
DA Bureau of Animal Industry

Republic of the Philippines
OFFICE OF THE SECRETARY
Elliptical Road, Diliman
1100 Quezon City

09 February 2021

ENDORSEMENT

The National Livestock Program hereby endorses the African Swine Fever Virus Nanogold Biosensor Test Kits to the **Bureau of Agricultural Research** to be used in ASF-related researches and programs. The ASFv Nanogold Biosensor Test Kit is being developed by the Central Luzon State University and is being utilized by the Bantay ASF sa Barangay or BABay ASF Program of the Department of Agriculture.

RUTH S. MICLAT-SONACO, DVSM
Program Director, National Livestock Program

A food-secure Philippines

Republic of the Philippines
Department of Agriculture
BUREAU OF ANIMAL INDUSTRY
Visayas Ave., Diliman, Quezon City
GaBAI sa Pag-unlad ng Paghahayupan
(+632) 8528-2240   director@bai.gov.ph   www.bai.gov.ph   @bai.gov.ph

April 30, 2021

EDGAR A. ORDEN, PhD.
President
Central Luzon State University
Science City of Munoz,
Nueva Ecija

Device:	ASFV Nanogold Biosensor Test Kit
EUP Number:	EUA202101
Institution:	Central Luzon State University
Indication:	This test is authorized for the following indication of use:

Screening test that qualitatively detects nucleic acid from ASFV in environmental samples such as surface swabs collected from pig pens and premises; water samples of farms; and fecal samples as an adjunct test for repopulation.

Authorized Laboratory: Emergency use of this test is limited to government testing

DEPLOYMENT

DA LAUNCHES INSPIRE, BABAY ASF IN BATANGAS; AWARDS P108M WORTH OF LIVESTOCK INTERVENTIONS TO CALABARZON PROVETs, FCAs, SUCs

DEPARTMENT OF AGRICULTURE (DA) LAUNCHED THE P28.1-BILLION INTEGRATED NATIONAL SWINE PRODUCTION INITIATIVES FOR RECOVERY AND EXPANSION (INSPIRE) PROGRAM AND THE P1.5-BILLION BANTAY AFRICAN SWINE FEVER (ASF) SA BARANGGAY (BABAY ASF) AIMED AT STRENGTHENING ITS ARTILLERY AGAINST ASF VIRUS. HELD AT DREAM ZONE, PROVINCIAL CAPITOL, BATANGAS CITY ON FEBRUARY 11, 2021.

DA4CALABARZON | DA4_CALABARZON | DEPARTMENT OF AGRICULTURE REGION IV-A | WWW.RFO4A.DA.GOV.PH

Dream Zone, Capitol Grounds, Batangas City | February 11, 2021

"Andali" in BABay ASF Surveillance

Key Elements:
1. Farm & Local area Identified
2. Decontaminate
3. Bioassay
4. Biosecurity Training
5. Sentinel pigs are deployed & monitored
6. Farm Registration
7. Regular area surveillance & health monitoring of farm.

CLSU and UNAHCO DAVAO (Public-Private) partnership

TURN-OVER OF ASF TEST KITS AND LABORATORY EQUIPMENTS

SURVEILLANCE

Northern Mindanao

CLSU and WISIUM (Public-Private) partnership
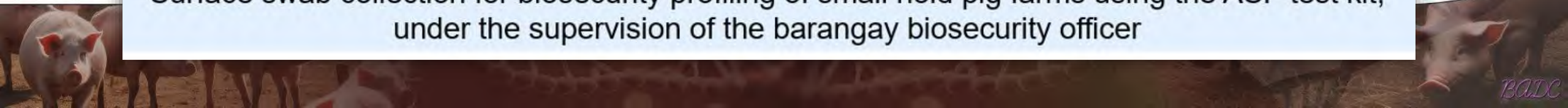
Backyard Farms

La Union

Bulacan

Quezon

Batangas

Surface swab collection for biosecurity profiling of small hold pig farms using the ASF test kit, under the supervision of the barangay biosecurity officer

Slaughterhouses and wet markets

Collection of other Samples for ASF Testing

Water sample

Raw pork meat

Blood Collection

Surface swab of hauling vehicles

Surface swab of hauling vehicles

DEPLOYMENT

KICK-OFF TESTING ACTIVITY USING THE ASF VIRUS NANOGOLD BIOSENSOR TEST KIT TECHNOLOGY

FOR FASTER AND COST-EFFECTIVE SURVEILLANCE AND DETECTION OF THE DREADED AFRICAN SWINE FEVER (ASF) IN BATANGAS PROVINCE

SIDC: The Largest Agri-based Cooperative in the Philippines

Gumagamit kami ng **ASF Nanogold Biosensor Test Kit** Madaling gamitin. Bago mag load ng baboy, Mag test muna. Mataas ang confidence namin na hindi kami magka-ASF.

Dr Kenneth Ibanez
SIDC Veterinarian

Coop Adoption

Test kits are being utilized by SUCs for research work by undergraduate vet-med and animal science thesis students

"Research Trigger Effect"

"Napakalaking tulong ng mga teknolohiya tulad ng ASF Biosensor dahil mahalagang malaman agad kung positive pa yung mga tinamaan ng ASF virus dati para makapag-repopulate agad at makabawi sa mga nalugi noon."

ANJO PEDERNAL
Farm Supervisor, Heptagon Farm, San Jose City, Nueva Ecija

Local Farm Adoption

Uses of ASFv Nanogold Biosensor

1. ASF screening test of new stocks

2. ASF surveillance & monitoring in sea/air entry ports & slaughter-houses

3. Farm's biosecurity appraisal before repopulation

4. Method for academic researches

The test kit assesses FREEDOM from….
1. Contamination in the farm, water, PPEs, farm tools, hauling vehicles, feeds
2. Infection in your herd
3. Contamination in raw and processed meat

….for one's peace of mind.

TAKE HOME MESSAGE!

By decentralizing ASF detection using the ASF Nanogold Biosensor test kit, every animal health practitioner and farm owner is empowered to do early detection towards a wise and timely implementation of control and preventive measures within the farm

Thank you

ACKNOWLEDGEMENT TO: