

POCKIT System

An Innovative Point-of-need PCR System

Nina Chen

Product Specialist from GeneReach

December, 6, 2018

Outline

- **TiLV detection by POCKIT system**
- **Technology**
- **PCR application**
- **Pipe line of POCKIT system**
- **Summary**
- **Operation Guide (hands-on-time)**

Overview of TiLV detection by POCKIT system

PetNAD nucleic acid co-prep kit

IQ plus TiLV kit on POCKIT micro plus



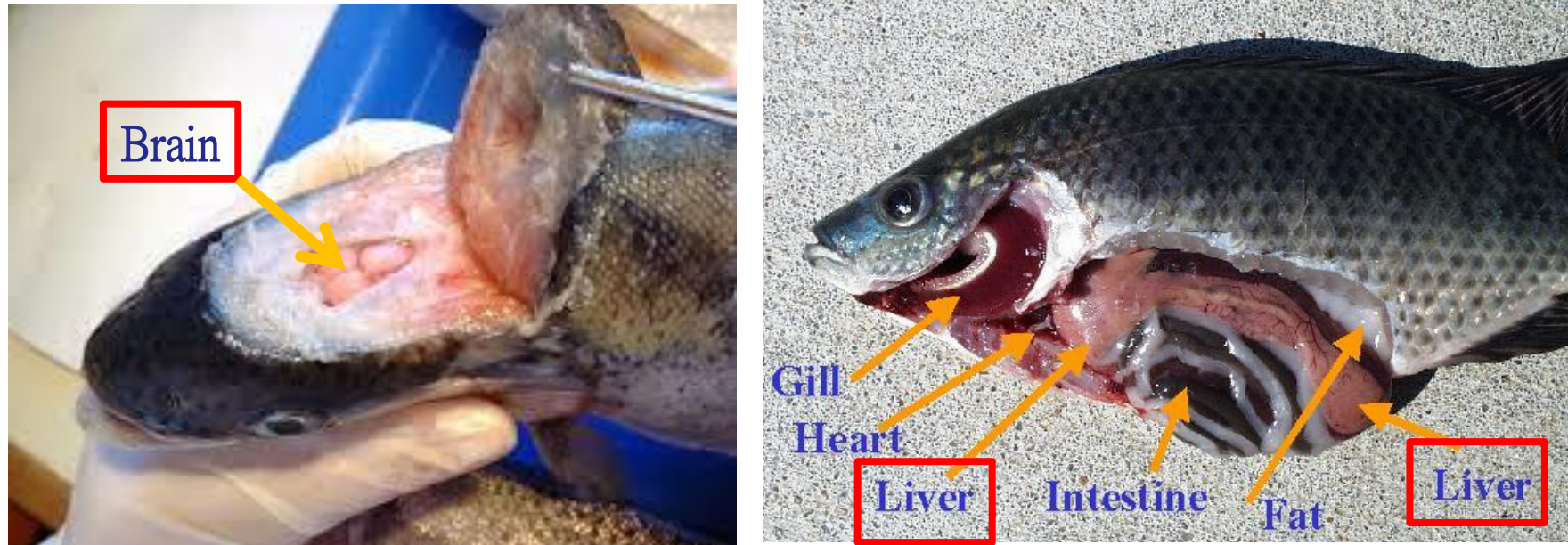
15-20 mins

42 mins

Content

- Sampling
- Nucleic Acid extraction system
 - PetNAD Nucleic Acid Co-prep kit
- PCR reaction
 - POCKIT micro plus
 - IQ plus TiLV kit

Sampling



- Sample type: Liver, Brain (or other potential source.)
- Fresh or Frozen sample

Sample preparation

40mg tissue + 500 ul PBS



Grinding



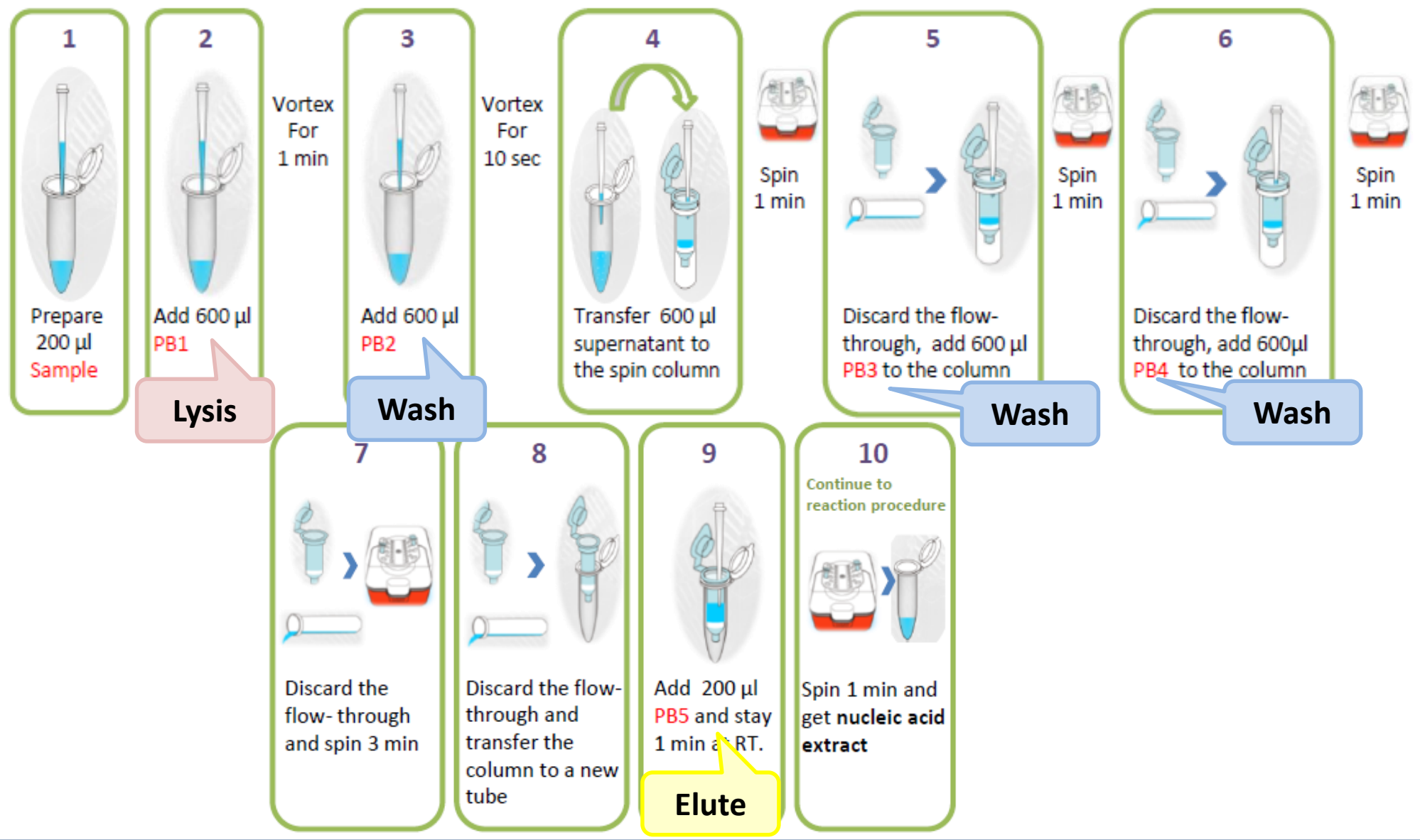
Centrifuge

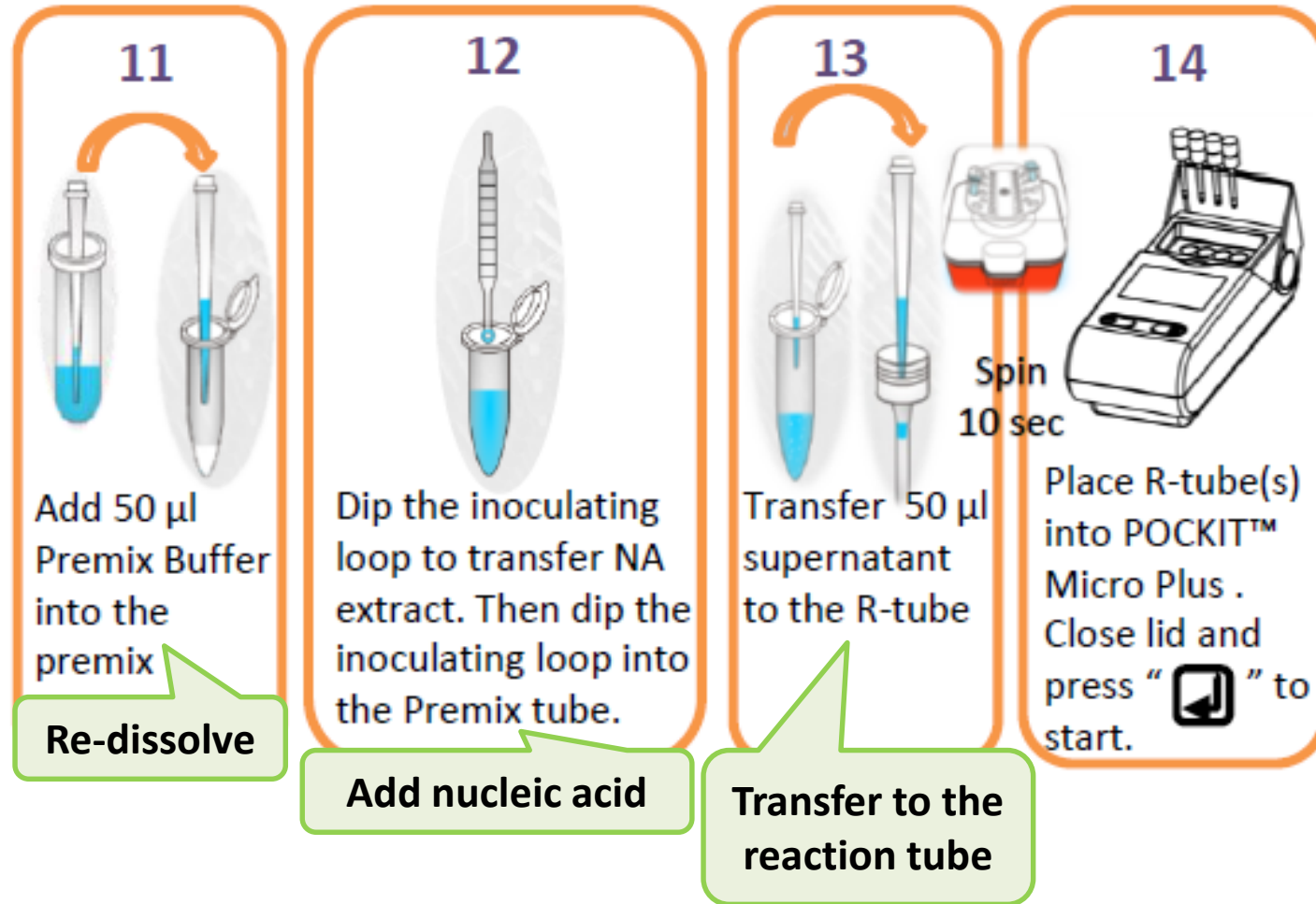
(12000g for 3 min; cubee for 5 min)



200ul supernatant
for nucleic acid extraction

PetNAD™ Nucleic Acid Co-prep Kit Operation steps:





PetNAD Nucleic Acid Co-prep Kit

Product Introduction



Item	Volume	Note: Before first use
PB1	36 ml/bottle, 1 bottle	
PB2	1 ml/bottle, 1 bottle	Add 35 ml 95% ethanol before use
PB3	20 ml/bottle, 1 bottle	Add 20 ml 95% ethanol before use
PB4	15 ml/bottle, 1 bottle	Add 25 ml 95% ethanol before use
PB5	3 ml/bottle, 1 bottle	
Spin Column & Collection Tube	50 sets/bag, 1 bag	
User Manual	1 copy	

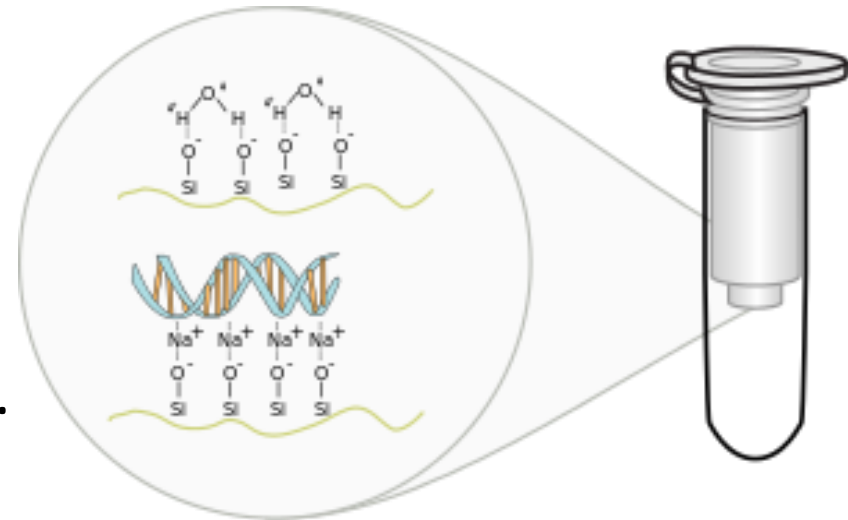
(50 tests/kit)

Note: Before first use, please add appropriate volume of ethanol as indicated.

- All reagents should be kept sealed tightly in cool and dry place at room temperature.
- Shelf life : 2 years

Principles of Nucleic Acid Co-prep Kit

- The nucleic acid is tightly bound on silica membrane under certain salt condition, and extensive washing removes all contaminations.

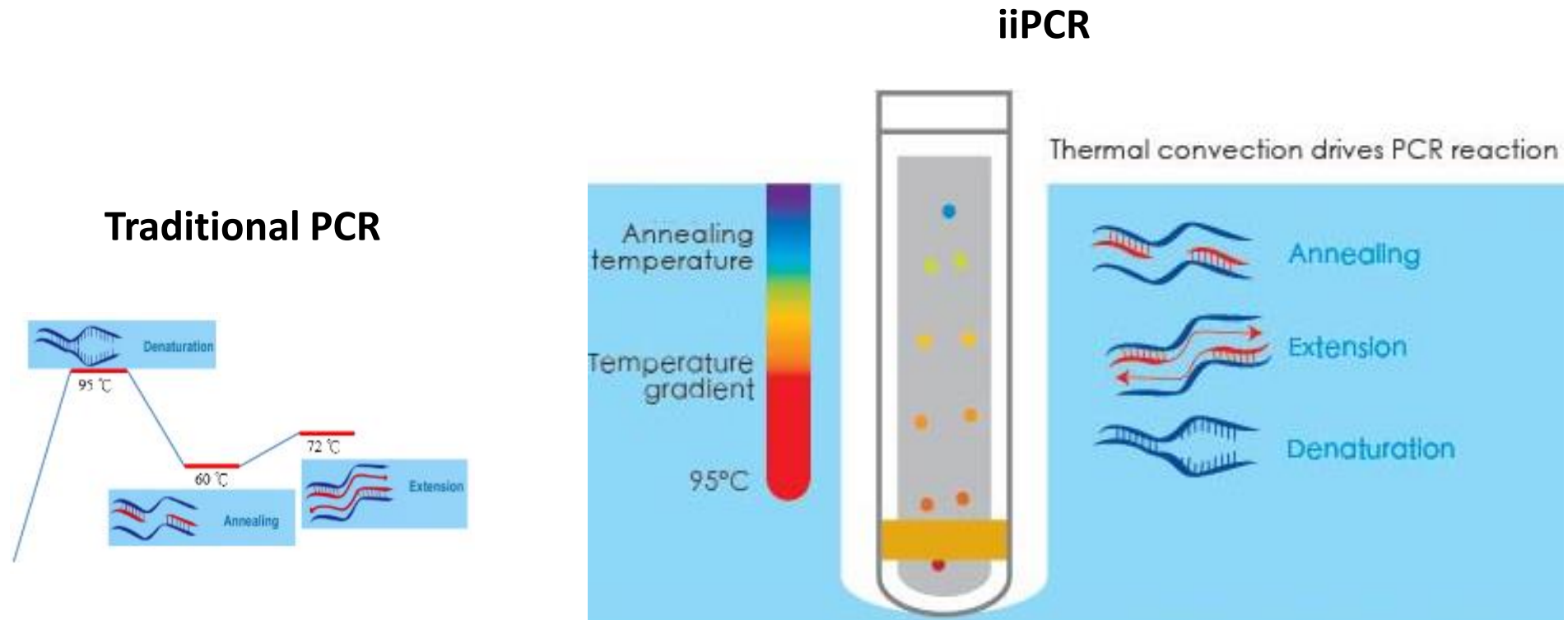


POCKIT micro plus

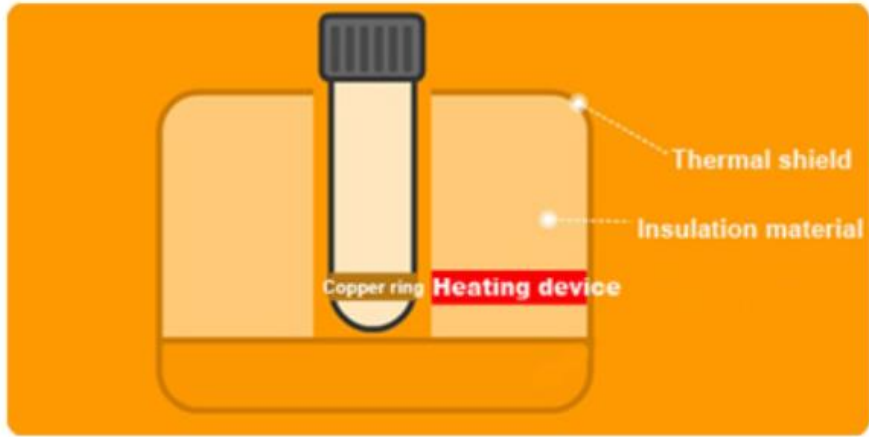


Specification	
Fluorescent Wavelength	520 nm (FAM)
Detection Target	DNA / RNA
Throughput	1 - 4 samples per run
PCR Reaction Time	Approx. 45 minutes
Dimensions & Weight	152 (W) × 63 (D) × 50 (H) mm
Weight	380 g
Operating Temperature	15 - 35°C
Power Source	Li-ion polymer battery
Warranty Information	1-year limited warranty

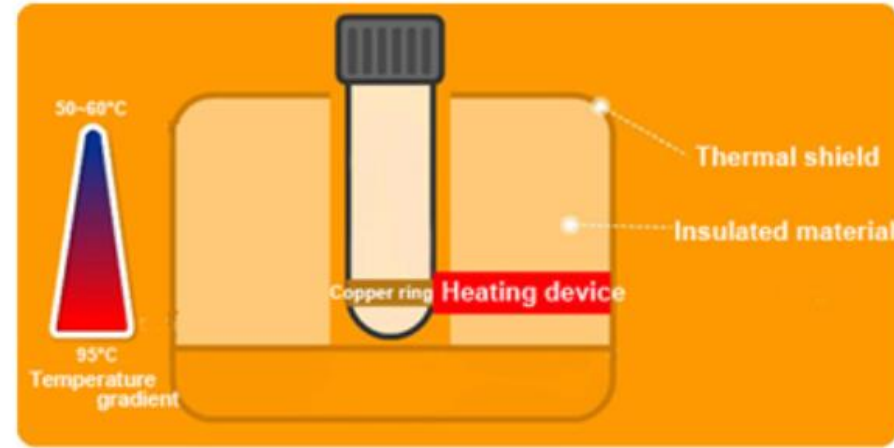
Insulated Isothermal PCR (iiPCR)



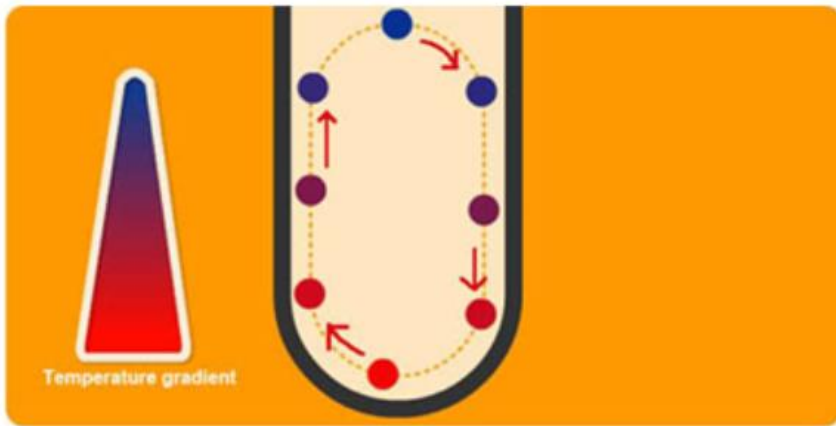
1 The environment is insulated by a radiation shell.



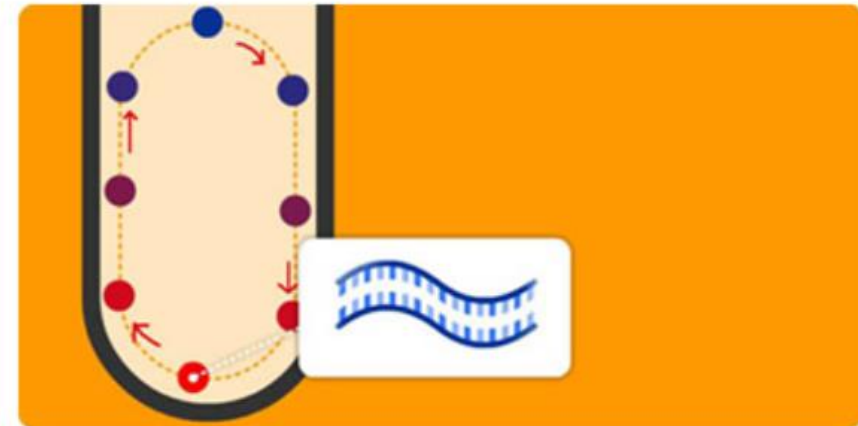
2 Natural thermal convection is induced by heat.



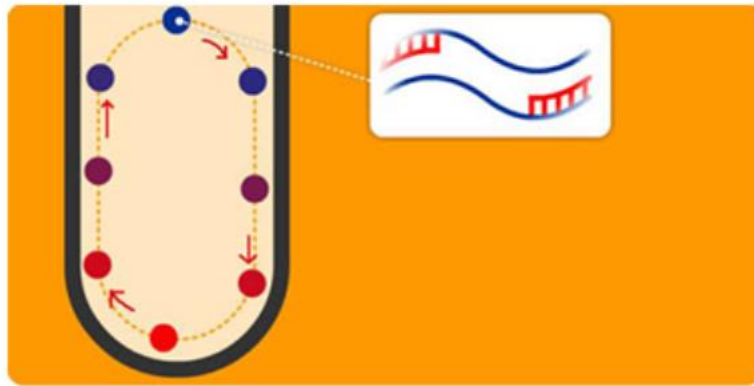
3 Thermal convection drives fluid cycling and results in PCR reaction.



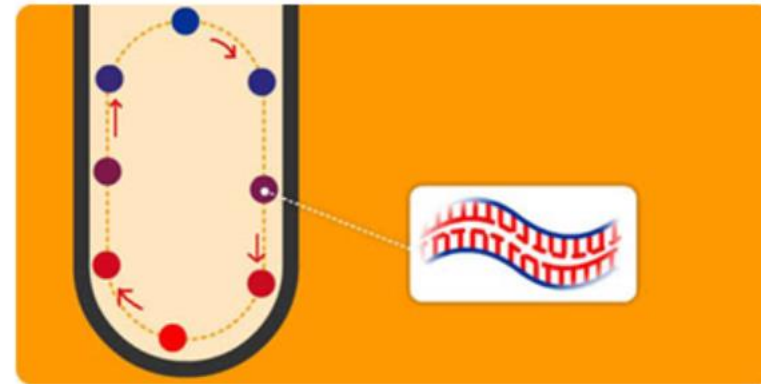
4 Denaturation of dsDNA



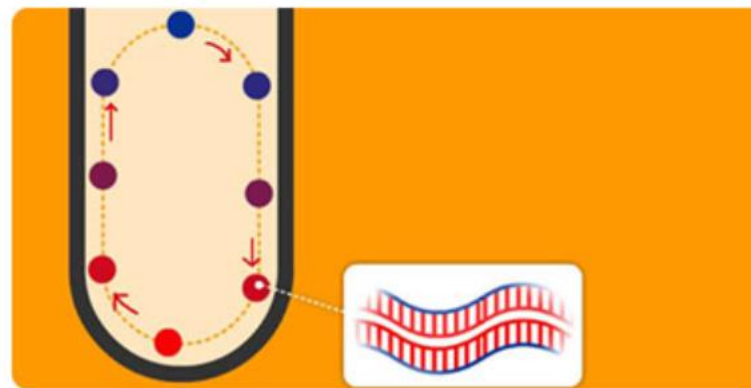
5 Primer annealing



6 Extension of the new strand



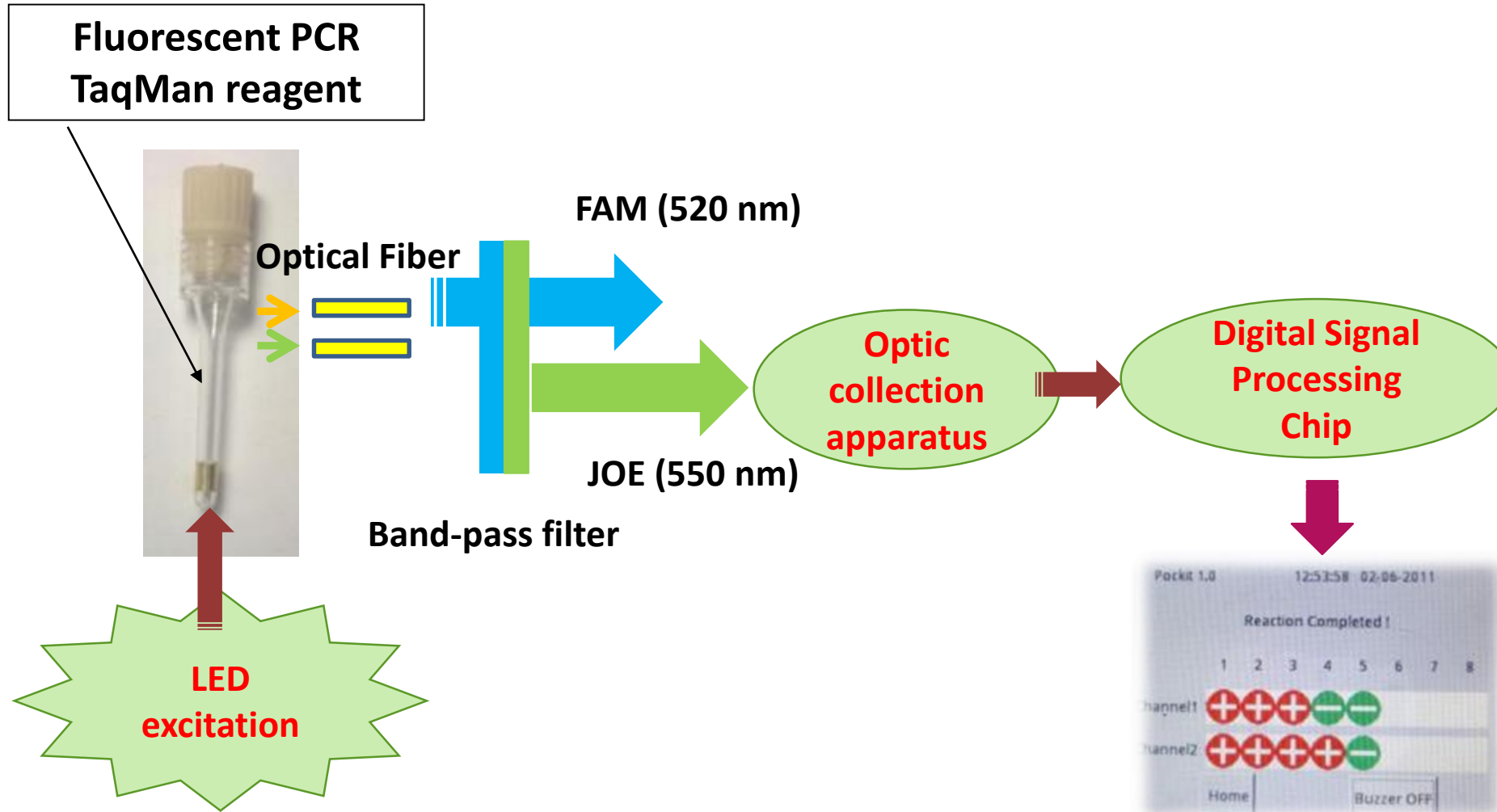
7 DNA is duplicated with the completion of one cycle of thermal convection.



Traditional PCR v.s. Insulated Isothermal PCR (iiPCR)

Traditional PCR	iiPCR
Time-consuming (usually > 1 h)	Time-saving (< 1 h)
Instrument is heavy	Light (Portable)
Complicated set-up	Single default program
Well trained personnel	No specific training is required

Automatic Data Interpretation Process



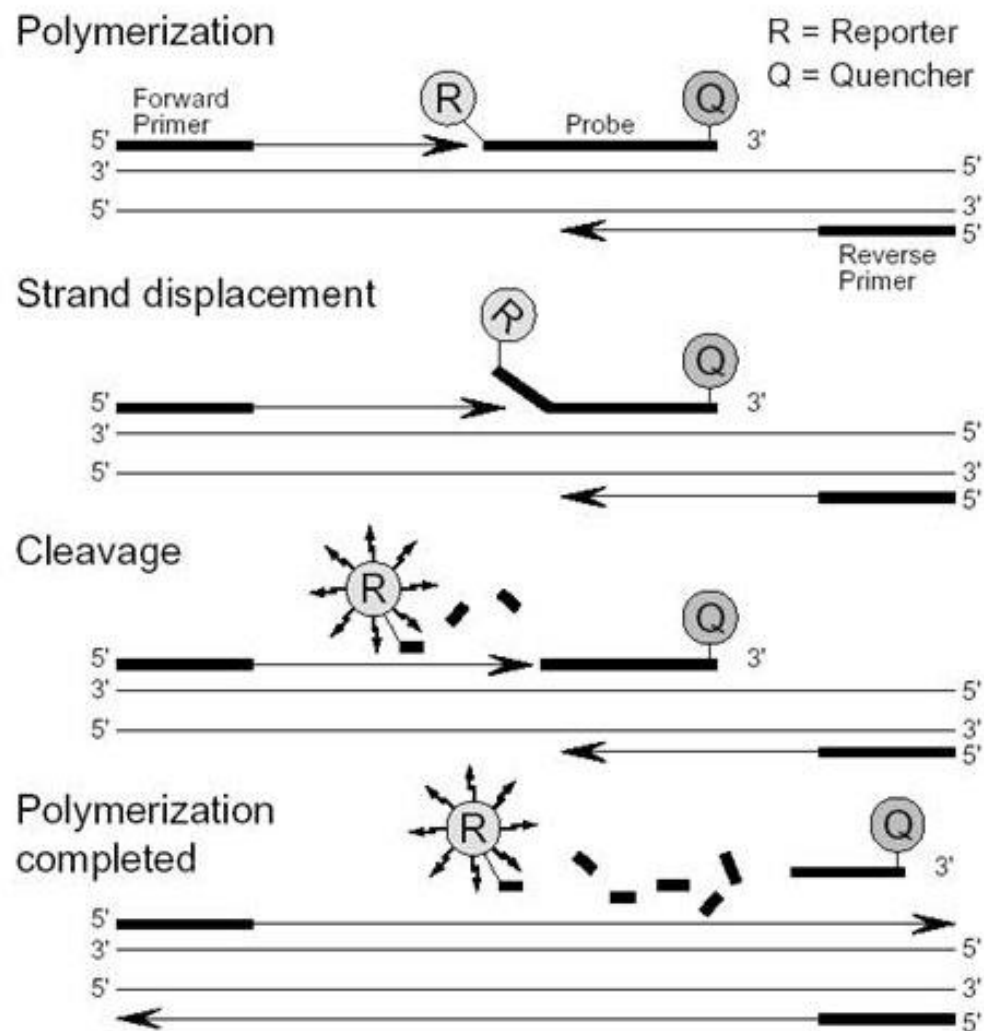
Signal Detection

TaqMan Probe

- Reporter (R)
- Quencher (Q)

Target sequence –
Fluorescence

No Target sequence –
No fluorescence



Reagent Format



- Lyophilized format
- Stored in 2-8°C (shelf life: 2 years after manufacturing)
- Can be transported in ambient temperature
- Open-bag stability: 2-8°C for 2 months or -20°C for 6 months

IQ plus TiLV kit



Component	Contents or Purpose	Amount
Premix Pack	Each Premix vial contains lyophilized pellet, which consists mainly of dNTPs, specific primers, fluorescent probes, and enzymes.	6 individually sealed zip-lock packs, 8 vials/pack
Premix Buffer	Reaction buffer to re-dissolve the lyophilized pellet.	2 vials, 1.3 ml/vial
P(+) Standard	Dry plasmid pellet.	1 vial
Standard Buffer	Reaction buffer to re-dissolve the P(+) Standard.	1 vial, 110 µl/vial
Inoculating Loops		3 packs(20pc/pack)

(48 tests/kit; R-tube ,48 tubes/pack)

- The kit should be stored at 4°C (shelf life : 3 years)
- 2 week short-term transportation will not affect the efficiency.
- Once the Premix Pack is opened, it would remain stable at 4°C for 2 months or at -20°C for 6 months.

IQ Plus TiLV Reagent Set

- Pathogen: TiLV (ss(-) RNA virus)
- Detected target: Segment 3
- Size of amplicon: 92bp
- Analytical sensitivity (95% hit rate by probit analysis)
 - LoD for POCKIT: up to 10 copies/rxn
- Specificity (Bioinformatic analysis)
 - Inclusivity: TiLV
 - Exclusivity: TLEV, VNN (in silico analysis)
- Interference test (with 300 ng/reaction genomic DNA): PASS
- Suggested sample type
 - brain tissue or liver tissue

Product Specification

- ❑ Product name: IQ Plus TiLV Reagent Set (48rxn/kit)
- ❑ Product format: iiPCR (single channel, 520 nm)
- ❑ Machine/POCKIT version : POCKIT Nucleic Acid Analyzer, POCKIT Micro Plus
- ❑ Intended user: fish farm & D-lab
- ❑ Applications: screening purpose, pathogen identification
- ❑ Suggested extraction Kit:
 - PetNAD™ Nucleic acid Co-prep Kit
 - taco™ mini Automatic Nucleic Acid Extraction System
- ❑ Suggested sample type: brain tissue or liver tissue

Advantages of iiPCR Detection Reagent

- **Easy-to-use:** Reagents in lyophilized format
- **Uni-dose:** easy to operate
- **High sensitivity and specificity**
- **Dual platform application:** can be adapted for most
Real-Time PCR instruments
- **Affordable**



Emergency Centre for Transboundary Animal Disease (ECTAD) - Viet Nam



Programme Overview

Components

News

Resources

Events

Partners

Using innovative technology for quicker results and quicker response



09/05/2017 Swiftly locating and identifying viruses at the source is the first step for a quick and smart response towards a disease outbreak. With the number of animal and human cases of influenza A (H7N9) outbreak in China drastically increasing, it is critical for Viet Nam to quickly monitor and detect any unusual virus emergence to prevent the intrusion of this virus and minimize its impacts.

A novel technology called Pen-side PCR has become available to enable this task. It is a surveillance method to detect viruses close to sampling sites like markets or district veterinary stations with a hand-held, battery-operated device. With this system, animal health experts will be able to detect and identify the virus within 2 hours after sampling, whereas the traditional real-time PCR would take in average of 2.5 days to get the result due to

transportation reasons.

Potential Applications of POCKIT system



Aquaculture



Companion
Animals



Livestock &
Poultry



IVD



Food Safety



Bio-security;
Surveillance program

Product list

	Aquaculture		Companion animal		Livestock				Food		Biothreat	Human Diseases		
	Shrimp	Fish	Canine	Feline	Equine	Bovine	Poultry	Swine	Ruminant	Microorganism	GMO & food adulteration	Biothreat	Human Diseases	
Available Reagents	AHPND/EMS Plasmid	Carp Edema Virus (CEV)	<i>Anaplasma platys</i>	<i>Bordetella bronchiseptica</i>	<i>Anaplasma phagocytophilum</i>	Bovine Leukemia Virus	Avian Reovirus (Asia & America regions only)	<i>Actinobacillus Pleuropneumonia</i>	Bluetongue Virus	<i>Cronobacter</i> spp.	GMO-CaMV 35S	<i>Bacillus anthracis</i> PL3	Chagas Disease	
	AHPND/EMS Toxin 1	IRIDO-Megalocytivirus	<i>Babesia gibsoni</i>	<i>Candidatus Mycoplasma haemominutum</i>	EAV	Bovine Tuberculosis	Chicken infectious anemia virus	Africa Swine Fever Virus	<i>Brucella abortus</i>	<i>Escherichia coli</i> (O157)	GMO-NOS	<i>Bacillus anthracis</i> pXO1	Chikungunya virus	
	<i>Baculovirus penaei</i>	IRIDO-Ranaviruses	<i>Bordetella bronchiseptica</i>	<i>Candidatus Mycoplasma turicensis</i>	EHV-1	<i>Brucella abortus</i>	Infectious bronchitis virus	Brachyspira hyodysenteriae	<i>Brucella melitensis</i>	<i>Listeria monocytogenes</i>	GMO-RRS	<i>Bacillus anthracis</i> pXO2	Dengue Virus (universal)	
	CMNV	ISAV	Canine Adeno Virus 2	<i>Chlamydophila felis</i>	EHV-3	<i>Brucella</i> spp.	Infectious Bursal Disease Virus	CSFV	<i>Brucella</i> spp.	Meat Ingredient	Meat Ingredient	<i>Brucella abortus</i>	Filariasis (<i>Brugia</i> spp.)	
	EHP	KHV	Canine Babesia	<i>Cytauxzoon felis</i>	EHV-4	BVDV-1	Infectious Laryngotracheitis	<i>Mycoplasma suis</i>	FMDV	Porcine Ingredient	Porcine Ingredient	<i>Brucella melitensis</i>	Filariasis (<i>Wuchereria bancrofti</i>)	
	IHHNV	<i>Streptococcus iniae</i>	Canine Influenza Virus	FCoV	EIAV	BVDV-2	Influenza A	FMDV	PPRV	<i>Salmonella</i> spp.		<i>Brucella</i> spp.	MERS-CoV	
	IMNV	SVCV	Canine Leishmaniasis	FCV	Influenza H3N8	<i>Campylobacter fetus</i>	Influenza H5 (Asian Lineage)	<i>Lawsonia intracellularis</i>		<i>Staphylococcus aureus</i>			Chagas disease	<i>Mycoplasma pneumoniae</i>
	MrNV	Tilapia Lake Virus (TiLV)	CDV	Feline Parvovirus	<i>Leptospira lipL32</i>	Rotavirus	Influenza H5 (American Lineage)	<i>Mycoplasma hyopneumoniae</i>		<i>Streptococcus agalactiae</i>			FV3 Rana Virus	Yellow Fever Virus
	NHPB	VHSV	CHV	FeLV	Rotavirus	<i>Tritrichomonas foetus</i>	Influenza H7	<i>Mycoplasma suis</i>					MERS-CoV	Zaire Ebolavirus
	PvNV	VNN	CPiV	FHV	<i>Salmonella</i> spp.		Influenza HP-H7 with insertion	PCV2					Mink enteritis virus	Zika Virus
	SHIV		CRCoV	FIV	<i>Streptococcus equi</i>		Influenza N9	PDCoV					<i>Paenibacillus larvae</i>	Leishmaniasis
	TSV		<i>E. granulosus / E. multilocularis</i>	Leptospirosis lipL32	<i>Taylorella equigenitalis</i> (CEM)		Marek's Disease	PEDV					<i>R. communis</i> (Ricin)	Leptospirosis
	<i>Vibrio harveyi</i>		<i>Ehrlichia canis</i>	<i>Mycoplasma felis</i>			<i>Mycoplasma gallisepticum</i>	Porcine parvovirus					Rift Valley Fever Virus	
	WSSV		Influenza H3N8	<i>Mycoplasma haemofelis</i>			<i>Mycoplasma synoviae</i>	PRRS-NA					<i>Salmonella</i> spp.	
	YHV		Leptospirosis lipL32	<i>Toxoplasma gondii</i>			NDV-class 1	PRRSV NADC30-like						
			Parvovirus				NDV-class 2	PRRSV-CN						
							NDV-Lasota	PRRSV-EU						
						<i>Salmonella</i> spp.	Pseudorabies Virus -gB gene							
						Avian Influenza H9	Pseudorabies Virus -gE gene							
							SVA							
							Swine Influenza Virus (Type A)							
Under development	MBV	Largemouth bass virus				Avian Eimeria Species	JEV						C. difficile	
						H7 (HPAI)	<i>Chlamydia psittaci</i>						Norovirus	
						H7 (LPAI)								

PCR applications in food production animals

Animal Quality Control



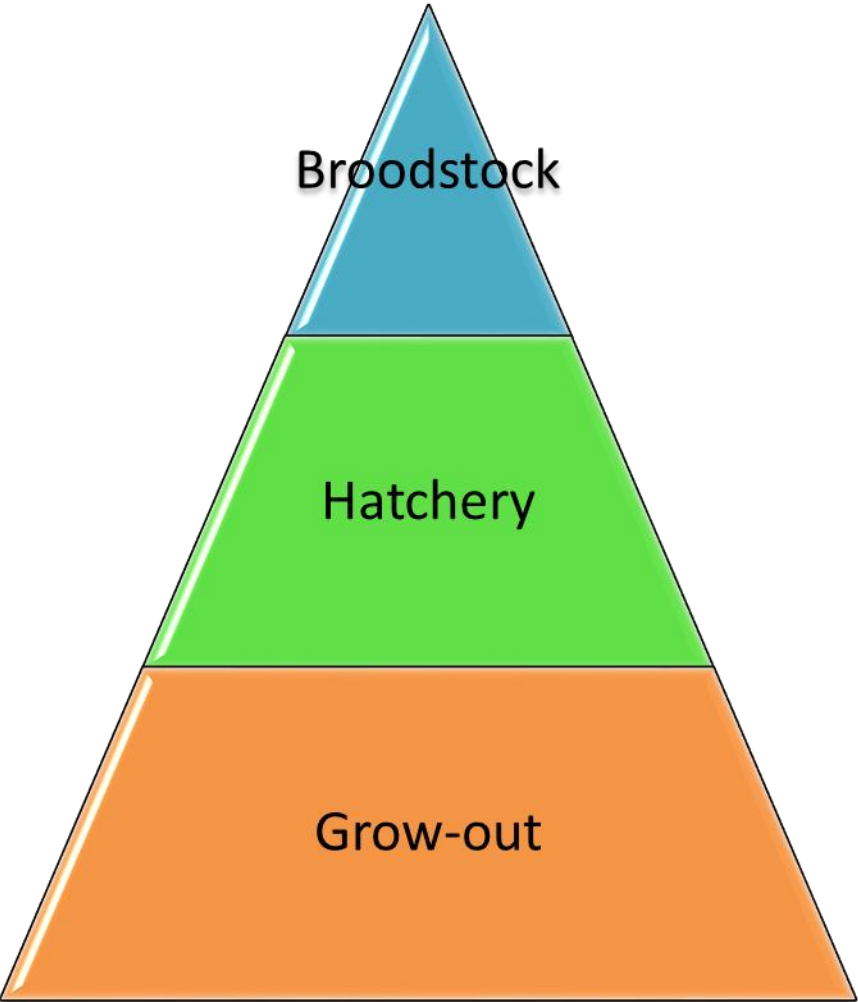
Early Detection



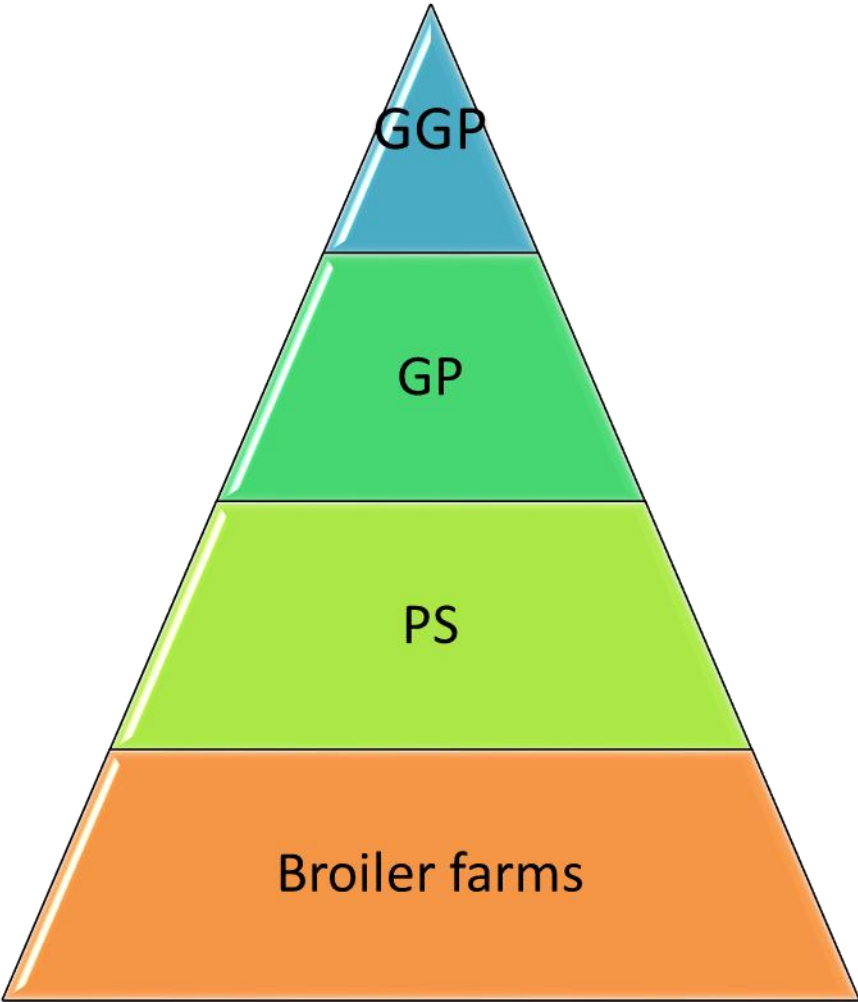
Biosecurity



Comparison between aquaculture and others

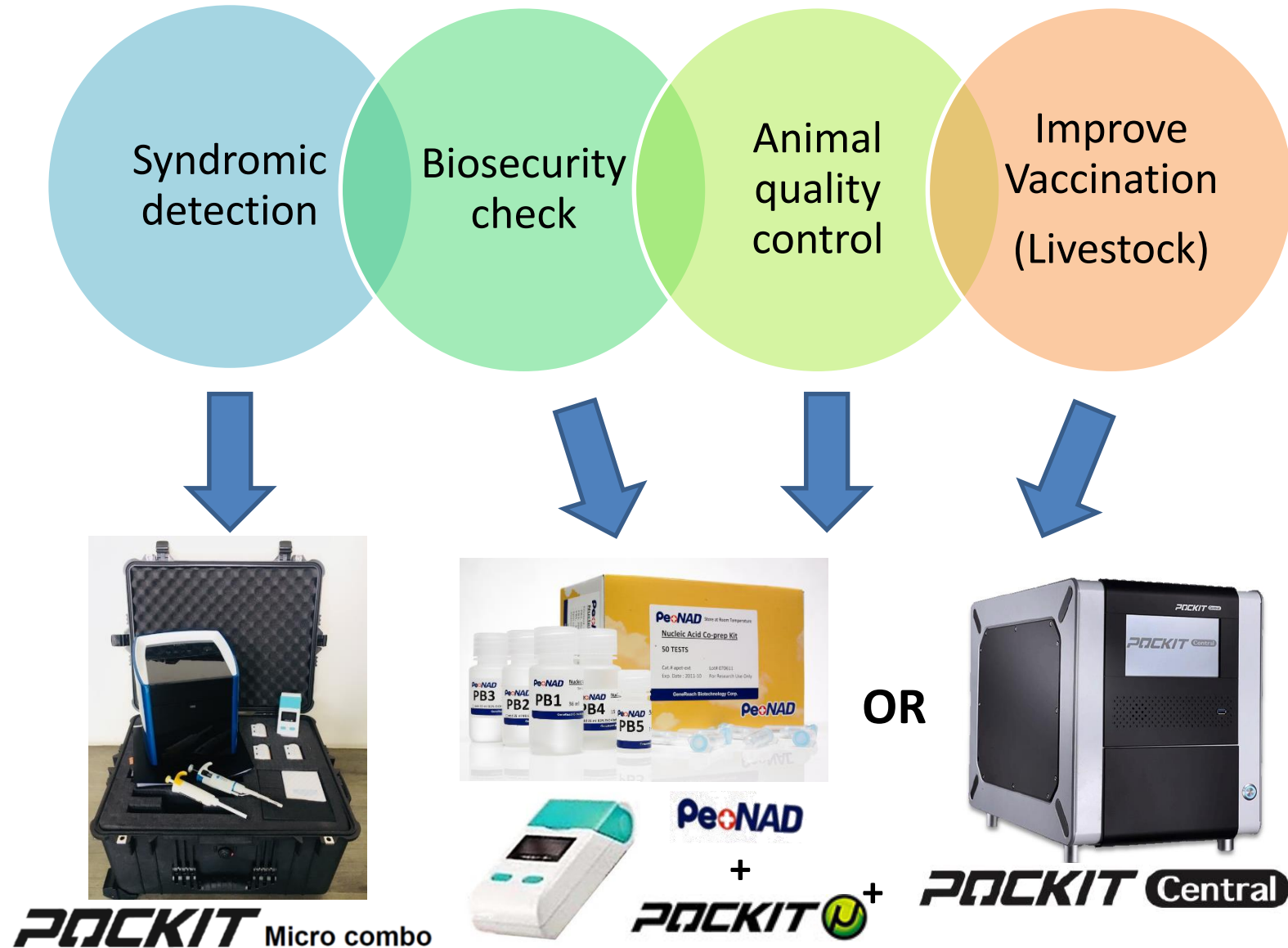


Shrimp farming



Broiler

Different POKKIT System for different application



Overview of TiLV detection by POCKIT system

PetNAD nucleic acid
co-prep kit

IQ plus TiLV kit on
POCKIT micro plus



**Nucleic Acid
Extraction**

**PCR
detection**



15-20 mins

42 mins

Overview of TiLV detection by POCKIT system



POCKIT Central

Sample in, result out fully automatic nucleic acid detection.



85 mins

Reaction Overview Operator:SCOTT Report No:201808170853

	Sample ID	Extraction Lot No.	Reagent Lot No.	Target	520	550
A	TEST 1	0206185	TFC0301185	TF	+	+
B	TEST 2	0206185	TFC0301185	TF	-	+
C	TEST 3	0206185	TFC0301185	TF	?	+
D	TEST 4	0206185	CDVA0307183	CDV	!	
E	TEST 5	0206185	CDVA0307183	CDV	-	
F	TEST 6	0206185	CDVA0307183	CDV	-	
G	TEST 7	0206185	CDVA0307183	CDV	-	
H	TEST 8	0206185	CDVA0307183	CDV	+	

UV Treatment Is Completed

Save to USB End

POCKIT Central – Sample in, result out fully automatic nucleic acid detection



Fully Automated Nucleic Acid Extraction & PCR System



Test report
•Positive/Negative

Reaction Overview Operator:SCOTT Report No:201808170853

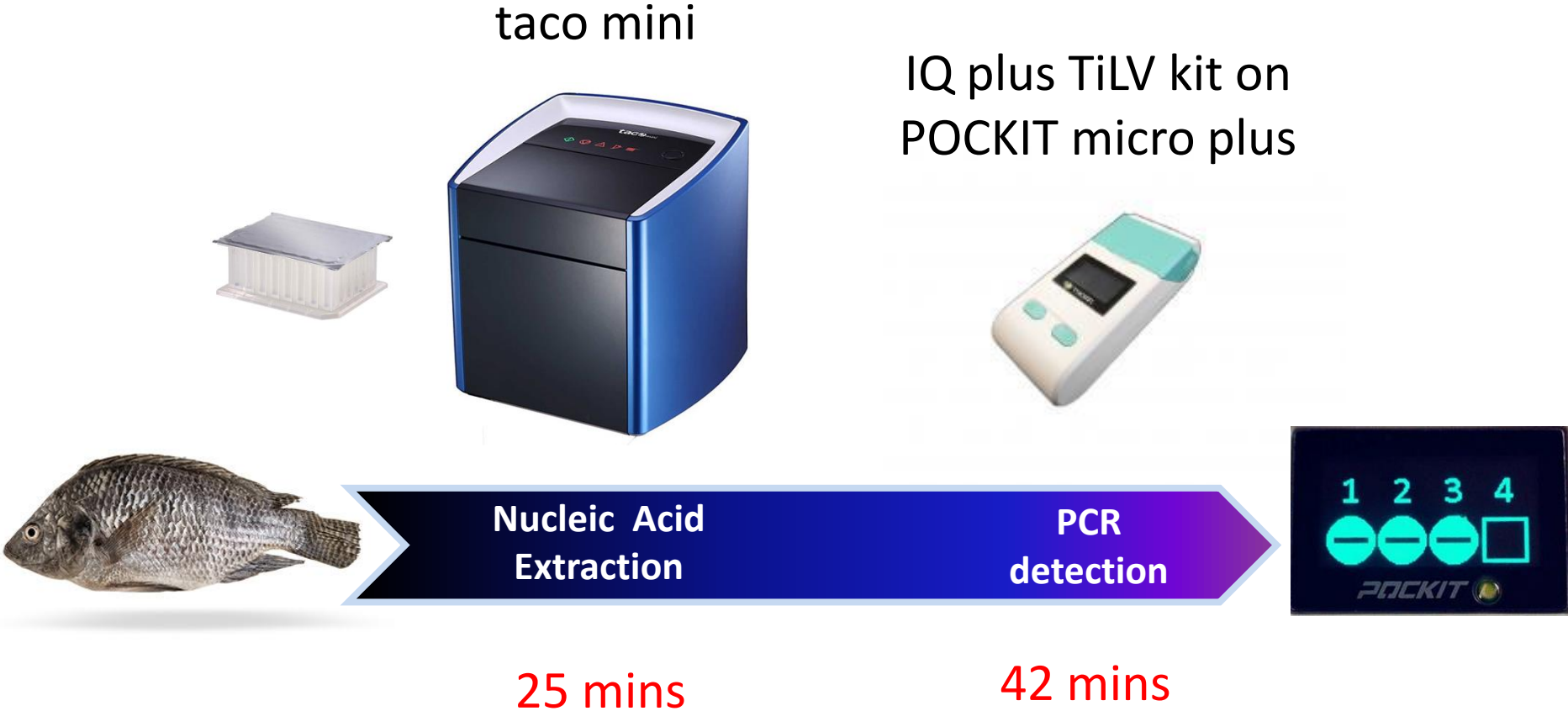
	Sample ID	Extraction Lot No.	Reagent Lot No.	Target	520	550
A	TEST 1	0206185	TFC0301185	TF	+	+
B	TEST 2	0206185	TFC0301185	TF	-	+
C	TEST 3	0206185	TFC0301185	TF	?	+
D	TEST 4	0206185	CDVA0307183	CDV	!	
E	TEST 5	0206185	CDVA0307183	CDV	-	
F	TEST 6	0206185	CDVA0307183	CDV	-	
G	TEST 7	0206185	CDVA0307183	CDV	-	
H	TEST 8	0206185	CDVA0307183	CDV	+	

UV Treatment Is Completed Save to USB End

SPECIFICATIONS	
Throughput	1 - 8 samples
Reaction Time	within 1.5 hours
Fluorescent Wavelength	520 nm & 550 nm
Dimensions (W x H x D)	310 x 400 x 480 mm
Weight	Approx. 21 kg

- Testing procedure of POCKIT Central**
1. Prepare the PCR reagent into Cartridge Set
 2. Open cover of pre-loaded extraction cartridge
 3. Add 200ul of sample to the first row
 4. Setup the information in POCKIT Central
 5. Run
 6. Wait for 85 minutes
 7. Read results (positive / negative)

Overview of TiLV detection by POCKIT system

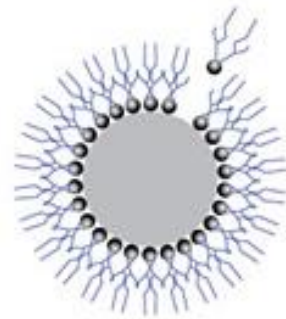




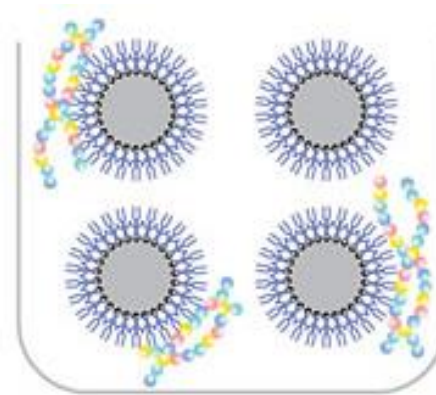
Specification	
Dimensions (W × D × H)	260 mm × 265 mm × 300 mm
Net Weight	5.5 kg
Power Requirement	1. 100-120V/ 200-240V AC, 50/60 Hz, 2A 2. Battery
Fuse	F2A/250V
Operating Temperature	16–30°C
Extraction Time	Within 30 minutes
Throughput	8 Samples

Principles and Advantages

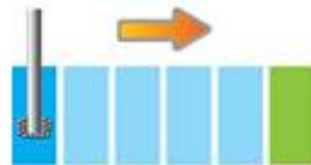
- The **taco**TM is based on the magnetic separation technology by motors and precision screw



magnetic beads are surface-modified.



The magnetic beads are capable to bind nucleic acids.



Break the cell in lysis buffer (deep blue). The nucleic acid from broken cell will be bound to the surface-modified magnetic beads.



Wash the beads for three times in wash buffer (light blue).



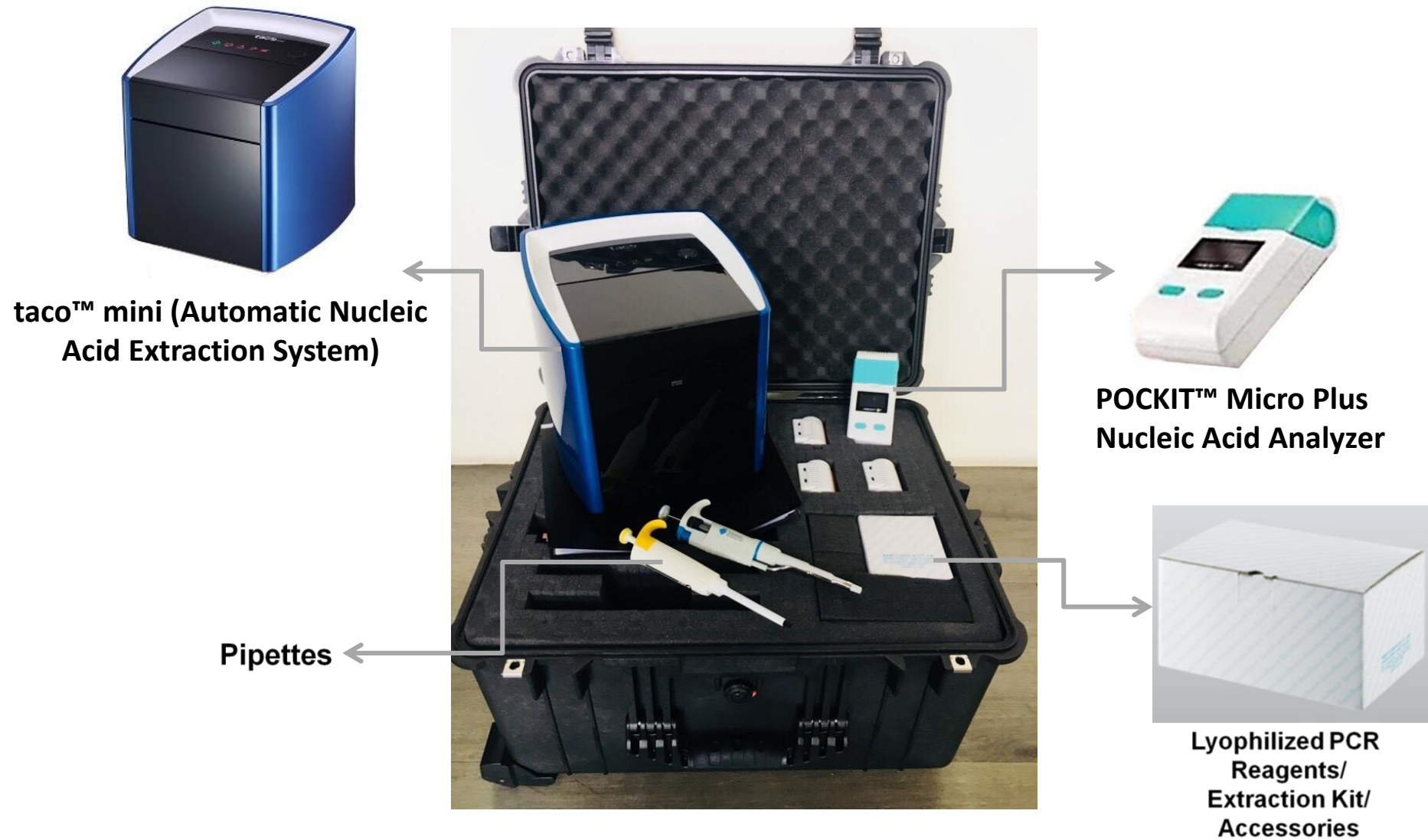
Release DNA from magnetic beads in the elute (green) and remove the beads with a magnetic bar.

Reagent and Sample Loading



Loading reagent Step	Reagents
1	Add Lysis Buffer with EtOH to column #1
2	Add 750 µl Washing Buffer A* to column #2
3	Add 750 µl Washing Buffer A to column #3
4	Add 750 µl Washing Buffer B* to column #4
5	Add 750 µl Washing Buffer B to column #5
6	Add 200 µl Eluting Buffer to column #6
7	Add 50 µl Magnetic Bead to column #2
8	Add Sample in column #1

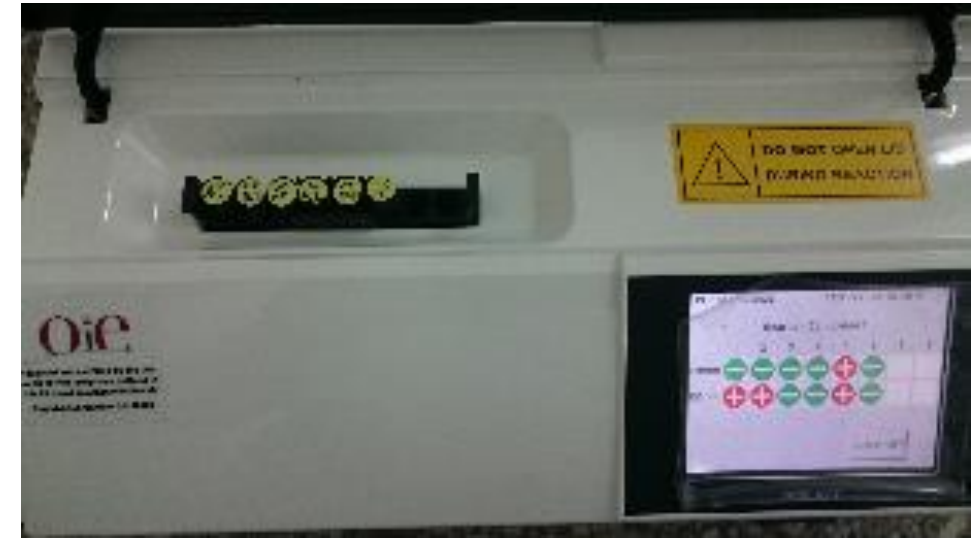
POCKIT Micro Combo System



Can farmers use PCR in the farm?



Small PCR lab in the farm



Check PLs



Publication



PLoS One. 2014; 9(3): e90645.

PMCID: PMC3953118

Published online 2014 Mar 13. doi: 10.1371/journal.pone.0090545

Validation of a Commercial Insulated Isothermal PCR-based POCKIT Test for Rapid and Easy Detection of White Spot Syndrome Virus Infection in *Litopenaeus vannamei*

[Yun-Long Tsai](#)¹, [Han-Ching Wang](#)², [Chu-Fang Lo](#)³, [Kathy Tang-Nelson](#)⁴, [Donald Lightner](#)⁴, [Bor-Rung Ou](#)⁵, [Ai-Ling Hour](#)⁶, [Chuan-Fu Tsai](#)¹, [Cheng-Chi Yen](#)¹, [Hsiao-Fen Grace Chang](#)¹, [Ping-Hua Teng](#)¹ and [Pei-Yu Lee](#)^{1,^}

WSSV iiPCR assay on POCKIT system Performance comparable to nested PCR (OIE approved)

	WSSV	Nested PCR		Total
		Positive	Negative	
iiPCR	Positive	374	9	383
	Negative	26	291	317
Total		400	300	700

- Sensitivity- 93.5%, Specificity- 97.0%; Agreement- 95.0% (kappa = 0.90)

PLoS One. 2014; 9(3): e90645.

Validation: Live stocks

Home / Journal of Food Protection® Number 8, August 2013, pp. 1308-1479



Detection of *Salmonella* in Chicken Meat by Insulated Isothermal PCR

Authors: Tsen, Hau-Yang¹; Shih, Chia-Ming¹; Teng, Ping-Hua²; Chen, Hsin-Yen³; Lin, Chia-Wai¹; Orlou, Chien-Shun⁴; Wang, Hwa-Tang Thomas²; Chang, Hsiao-Fen Grace²; Chung, Ta-Yu²; Lee, Pei-Yu²; Chiang, Yu-Cheng⁵

Salmonella spp 2013

Original Article

Insulated Isothermal Reverse Transcriptase PCR (iiRT-PCR) for Rapid and Sensitive Detection of Classical Swine Fever Virus

O. Lung^{1,*}, J. Pasick², M. Fisher², C. Buchanan¹, A. Erickson¹ and A. Ambagala¹

Article first published online: 27 JAN 2015
DOI: 10.1111/tbed.12318

© Her Majesty the Queen in Right of Canada 2015 Reproduced with the permission of the Minister of Health.



Issue

Transboundary and Emerging Diseases

Early View (Online Version of Record published before inclusion in an issue)

Chinese Swine Fever Virus 2015

Original Article

A Rapid Field-Deployable Reverse Transcription-Insulated Isothermal Polymerase Chain Reaction Assay for Sensitive and Specific Detection of Bluetongue Virus

A. Ambagala^{1,*}, S. Pahari¹, M. Fisher¹, P.-Y. A. Lee², J. Pasick³, E. N. Ostlund⁴, D. J. Johnson⁴ and O. Lung¹

Article first published online: 19 JUL 2015
DOI: 10.1111/tbed.12388

© Her Majesty the Queen in Right of Canada (2015). Reproduced with the permission of the Minister of Health of Canadian Food Inspection



Issue

Transboundary and Emerging Diseases

Early View (Online Version of Record published before inclusion in an issue)

Porcine epidemic diarrhea virus and Porcine delta coronavirus

2016 *Journal of Virological Methods* (accepted)

Validation: Pet animals

Wilkes et al. BMC Veterinary Research 2014, 10:213
http://www.biomedcentral.com/1746-6148/10/213



METHODOLOGY ARTICLE

Open Access

Rapid and sensitive detection of canine distemper virus by one-tube reverse transcription-insulated isothermal polymerase chain reaction

Rebecca P Wilkes^{1*}, Yun-Long Tsai², Pei-Yu Lee², Fu-Chun Lee², Hsiao-Fen Grace Chang² and Hwa-Tang Thomas Wang²

Canine distemper virus 2014



Short communication

An insulated isothermal PCR method on a field-deployable device for rapid and sensitive detection of canine parvovirus type 2 at points of need

Rebecca P. Wilkes^a, Pei-Yu A. Lee^b, Yun-Long Tsai^b, Chuan-Fu Tsai^b, Hsiu-Hui Chang^b, Hsiao-Fen G. Chang^b, Hwa-Tang T. Wang^b

Canine parvovirus 2015



Journal of Virological Methods

Volume 220, August 2015, Pages 35–38



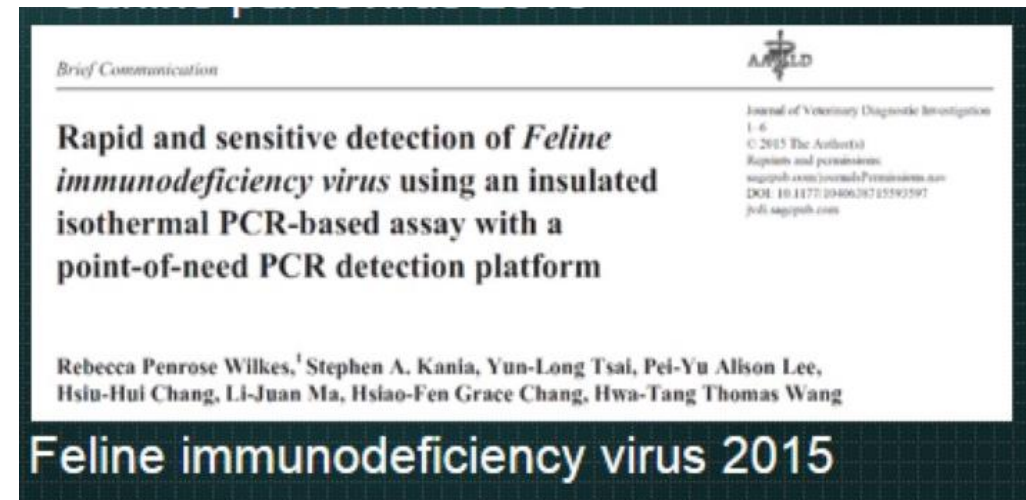
Short communication

An insulated isothermal PCR method on a field-deployable device for rapid and sensitive detection of canine parvovirus type 2 at points of need

Rebecca P. Wilkes^a, Pei-Yu A. Lee^b, Yun-Long Tsai^b, Chuan-Fu Tsai^b, Hsiu-Hui Chang^b, Hsiao-Fen G. Chang^b, Hwa-Tang T. Wang^b

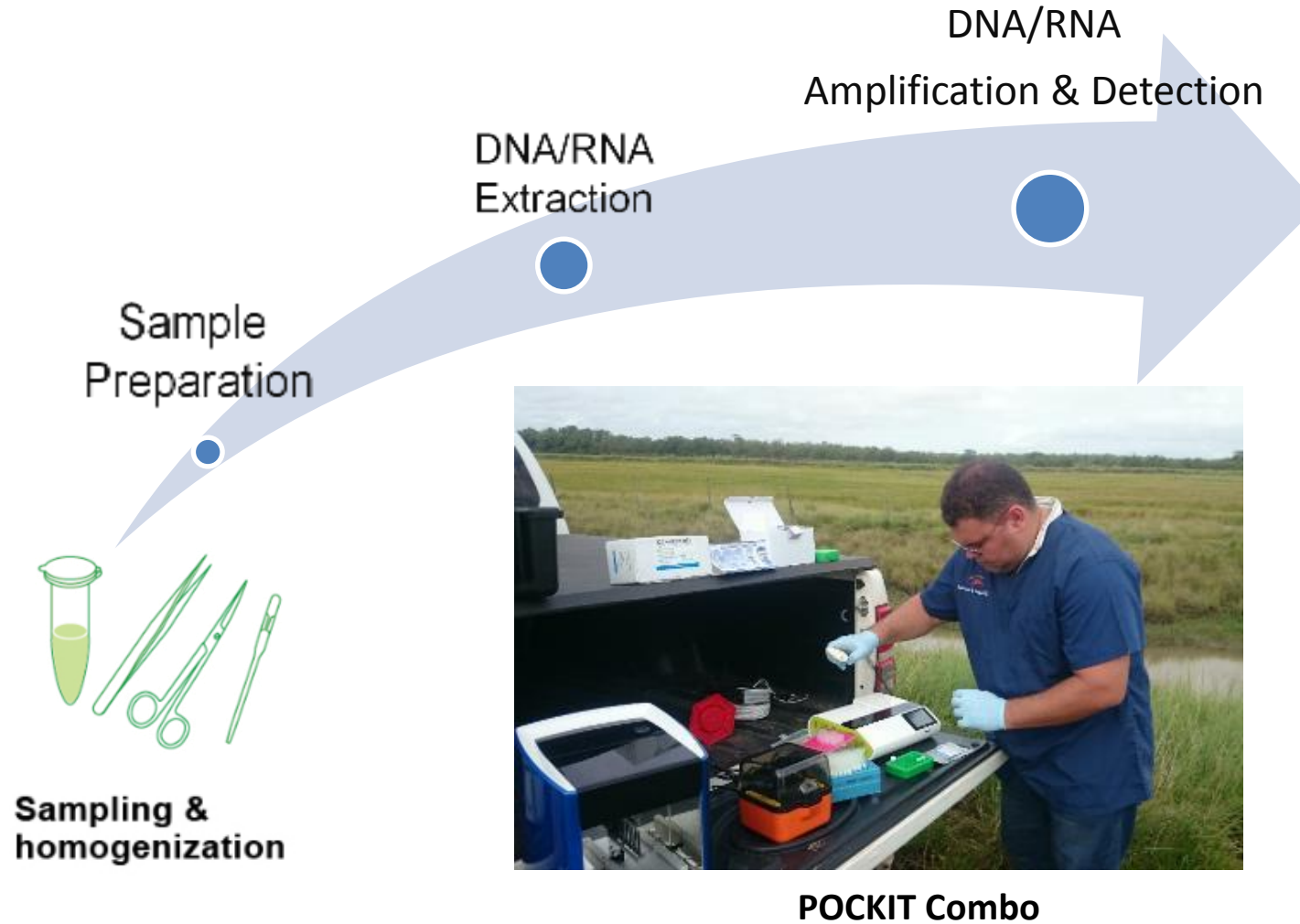
Under a Creative Commons license

Equine H3N8 influenza virus 2015



Feline immunodeficiency virus 2015

We are providing a Total Solution for Nucleic Acid Detection

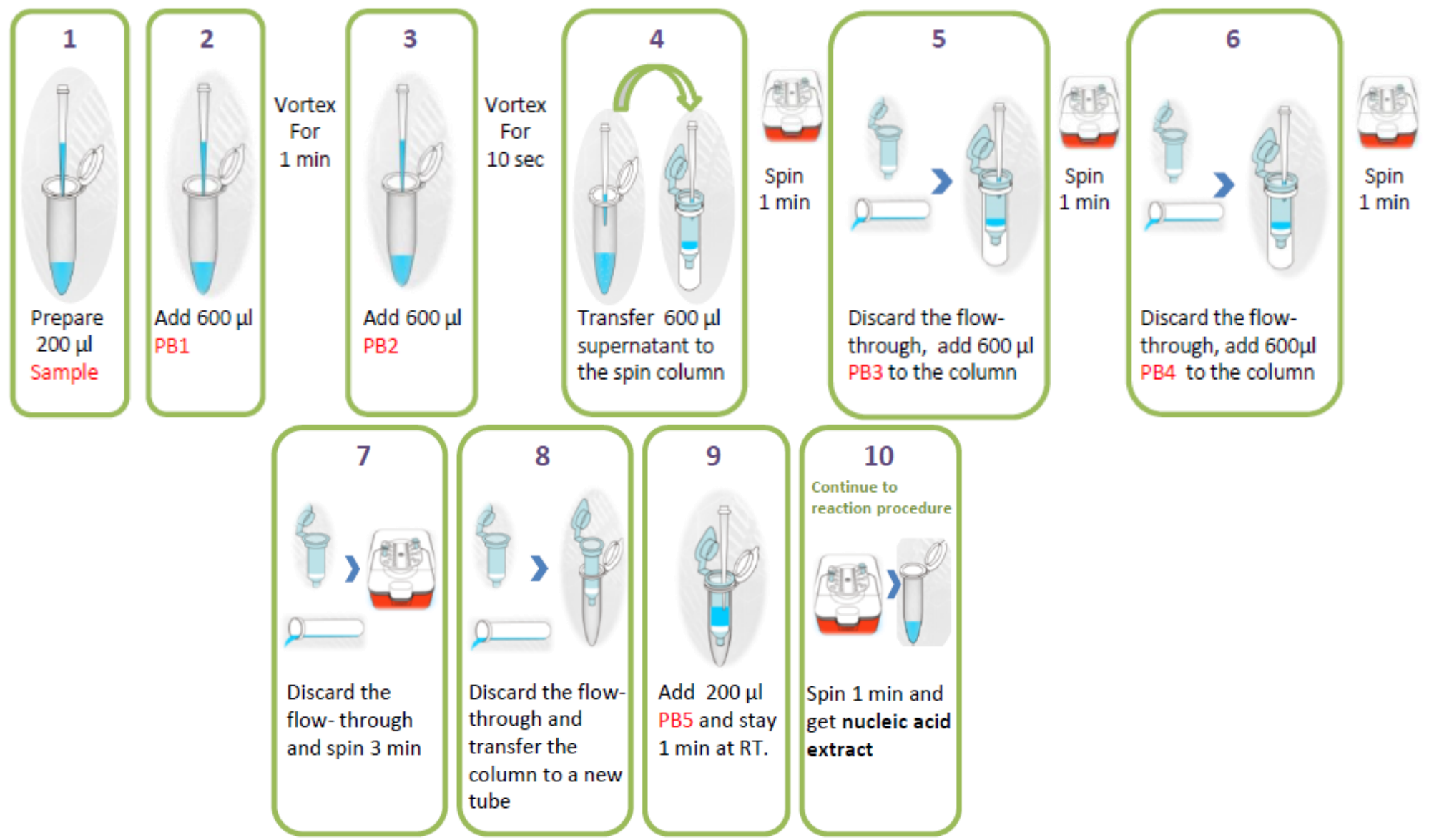


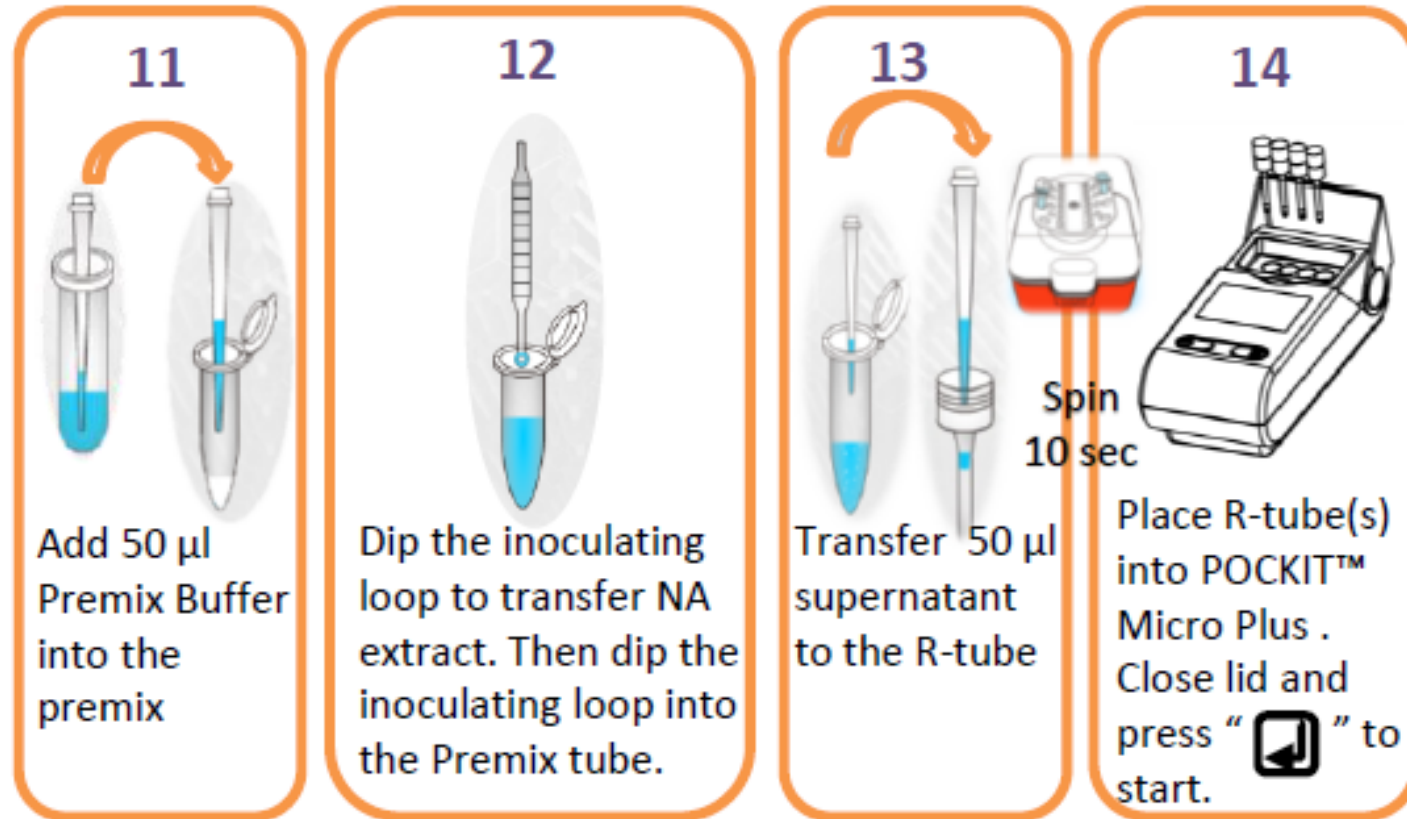
Reagents available

Shrimp	Fish
IQ Plus WSSV Kit with POCKIT System	IQ Plus Irido-Ranaviruses Kit
IQ Plus TSV Kit	IQ Plus VNN Kit
IQ Plus YHV Kit	IQ Plus KHV Kit
IQ Plus IMNV Kit	IQ Plus Irido-Megalocytivirus Kit
IQ Plus PvNV Kit	IQ Plus SVCV Reagent Set
IQ Plus IHHNV Kit	IQ Plus VHSV Reagent Set
IQ Plus NHPB Kit	IQ Plus CEV (Carp edema virus) Reagent Set
IQ Plus <i>V. harveyi</i> Kit	IQ Plus <i>Streptococcus iniae</i> Reagent Set
IQ Plus AHPND/EMS Toxin 1 Kit	IQ Plus TiLV Reagent Set
IQ Plus AHPND/EMS Plasmid Kit	IQ Plus IHNV Reagent Set
IQ Plus EHP Reagent Set	IQ Plus ISAV Reagent Set
IQ Plus CMNV Reagent Set	
IQ Plus Baculovirus penaei Reagent Set	
IQ Plus <i>Vibrio fluvialis</i> Reagent Set	
IQ Plus MrNV Reagent Set	
IQ Plus SHIV Reagent Set	

Hands-on-time

PetNAD™ Nucleic Acid Co-prep Kit Operation steps:

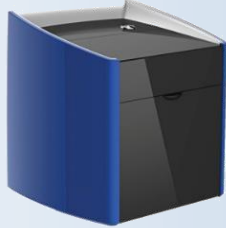




Thank you for your attention

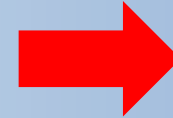
Pipe line of PCKIT

taco™ mini Automatic Nucleic Acid Extraction System



+

POCKIT Portable Nucleic Acid Analyzer



POCKIT combo

taco™ mini Automatic Nucleic Acid Extraction System



+

POCKIT Micro Hand-held Nucleic Acid Analyzer



POCKIT Micro combo

POCKIT Central

Sample in, result out fully automatic nucleic acid detection.



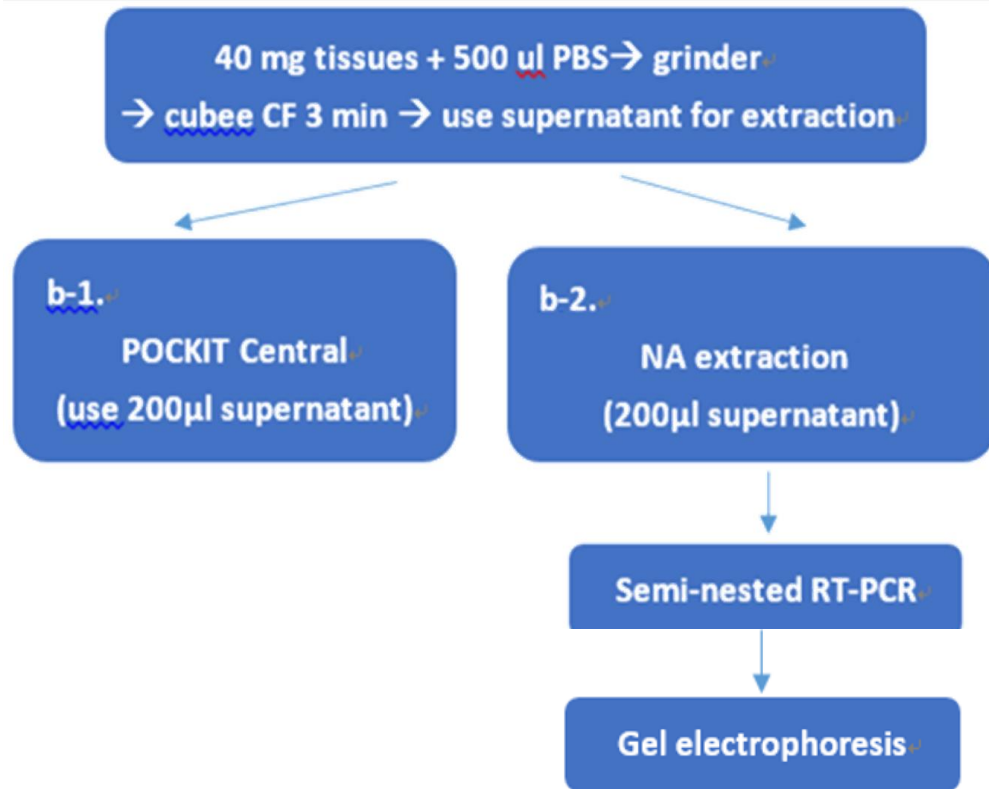
Analytical sensitivity of TiLV RT-iiPCR

Copy/rxn	hit rate	percentage
100	2/2	100%
30	20/20	100%
10	16/20	80%
NTC	0/26	0%

***serial dilutions of IVT RNA**

Limit of detection (LoD) 95% is 12 genome equivalents (probit analysis).

Performance of TiLV RT-iiPCR on POCKIT™ Central System



		Semi-nested RT-PCR		
		Positive	Negative	Total
TiLV RT-iiPCR	Positive	17	3	20
+POCKIT Central	Negative	1	71	72
Total		18	74	92

Agreement 95.65%; CI (95%): 90.73%~100%, k=0.87

Summary:

Higher than 95% agreement between RT-iiPCR and reference semi-nested RT-PCR

Sample number: 92 samples

Sample type: liver, brain, gill, muscle, mix