

Biosensor technology and its application in aquaculture

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Outlines

- Overview about monoclonal antibody and strip test
- Examples: WSSV-VP28
 - : dual strip tests; WSSV-YHV
 - : WSSV-ICP11
 - : IMNV
- MAb production against ToxA and ToxB of VP-AHPND

🚧 60% of disease losses in shrimp aquaculture have been caused by viral pathogens and 20% by bacterial pathogens. (Flegel; 2012)

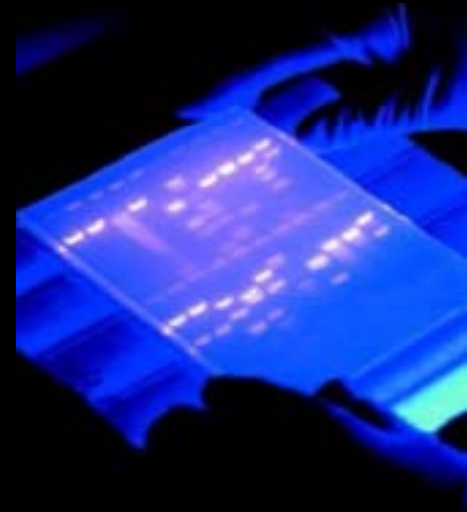
🚧 Two virus pathogens: (1) WSSV & (2) IMNV that have caused more than \$16 billion in losses combined since the first outbreaks. (Lightner; et al. 2012)



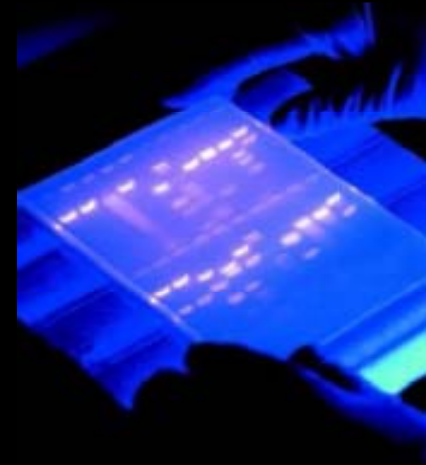
Detection of Shrimp Pathogens

Nucleic Based Detection

- Fast & easy to develop
- High sensitivity
- **Suitable for broodstock**
- Qualitative & quantitative detection
- One-step PCR, Nested PCR, Real-time PCR & LAMP

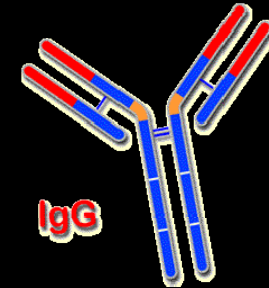


Nucleic Based Detection



- Disadvantageous features
- High prices
- Required well equipped laboratory
- skilled personnel
- Too sensitive: successful farming w/low infection

Immuno Based Detection

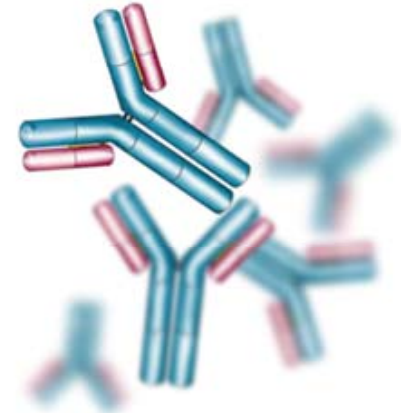


- Relatively Low cost/sample
- Large number of samples
- Medium sensitivity
- **Suitable for surveillance of disease during farming**
- Several types of assay including immunochromatographic strip test

Monoclonal Antibody

■ Advantageous Features

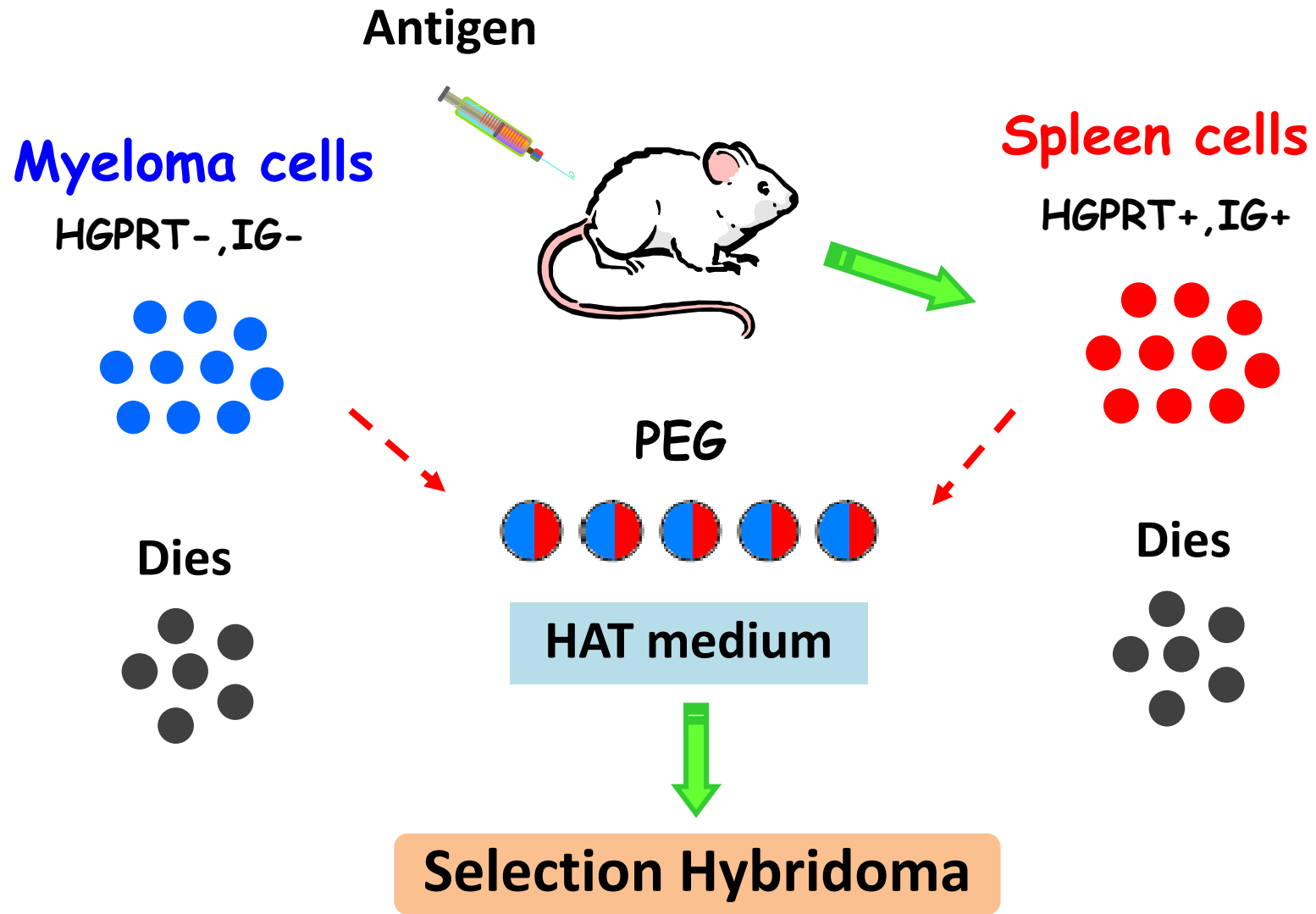
- Not Require highly Purified Antigen
- Homogeneous-Stable Quality
- Against One Epitope
- Unlimited Quantity



■ Disadvantageous Features

- Additional Cost of Production and Maintenance
- May not work in some assays

Monoclonal Antibody Production





Hybridoma Technique



MAb



Specificity test



Large scale

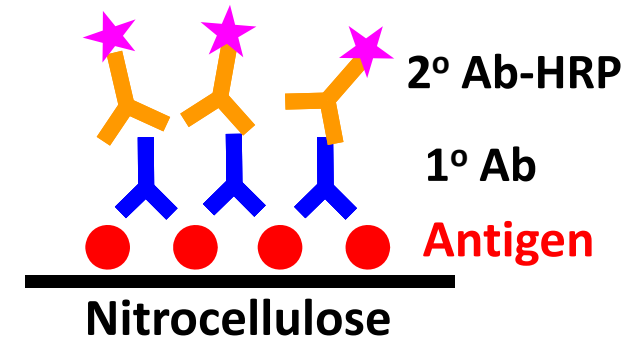


Purification

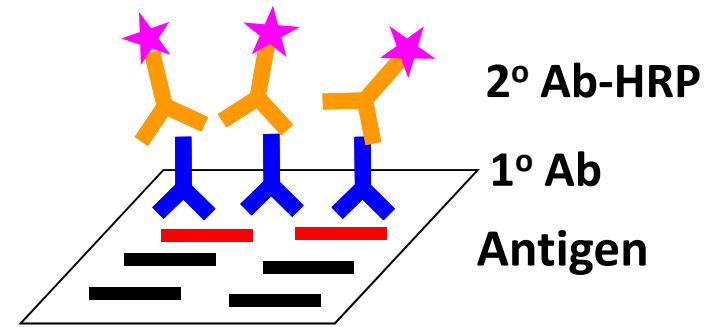


Affinity chromatography

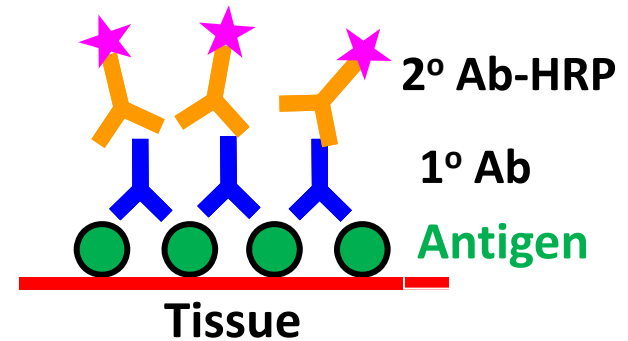
Dot blot



Western blot



IHC



Lateral Flow Immunochromatographic Strip (LFICS)

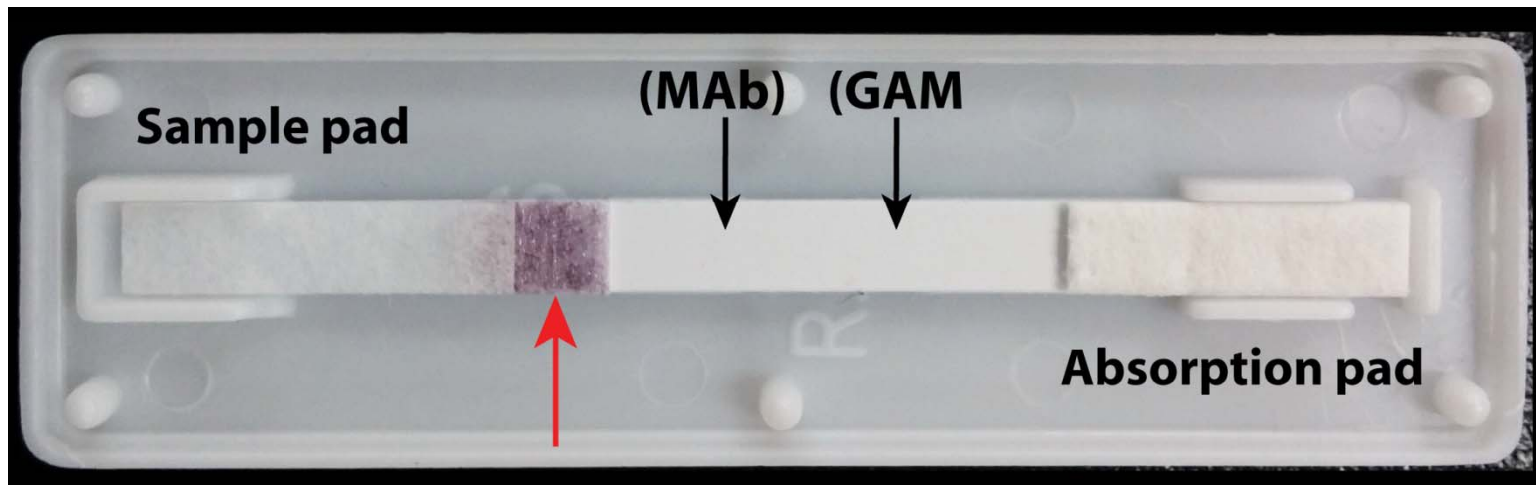
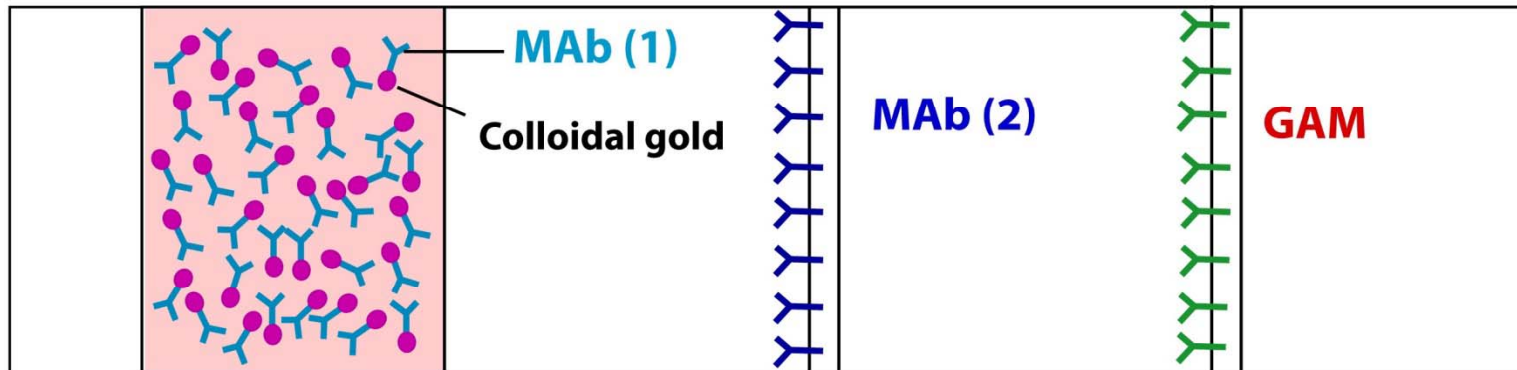
- Widely used in various field Diagnosis
- Simple, Low Cost and Rapid Detection
- Qualitative or Semiquantitative
- Low Signal Intensity- Poor Sensitivity
- Single Analyte Detection
- Improve Performance by Different Kinds of Nanoparticles

Development of LFICS

Large Antigen Detection: many epitopes

- Protein, Carbohydrate, Bacteria, Virus etc.
- Sandwich format: Requires two or more antibodies against different epitopes
- **One labeled antibody & capture antibody**

Development of Sandwich LFICS



**Glass fiber containing
MAb-colloidal gold**

Overall process

Protein Expression

Protein Purification

Immunization

Monoclonal Antibody Production

Selection and Screening of MAbs

Characterization of MAbs



DISEASES OF AQUATIC ORGANISMS (2006)

Vol. 72: 101–106, 2006

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published October 17

A simple and rapid immunochromatographic test strip for detection of white spot syndrome virus (WSSV) of shrimp

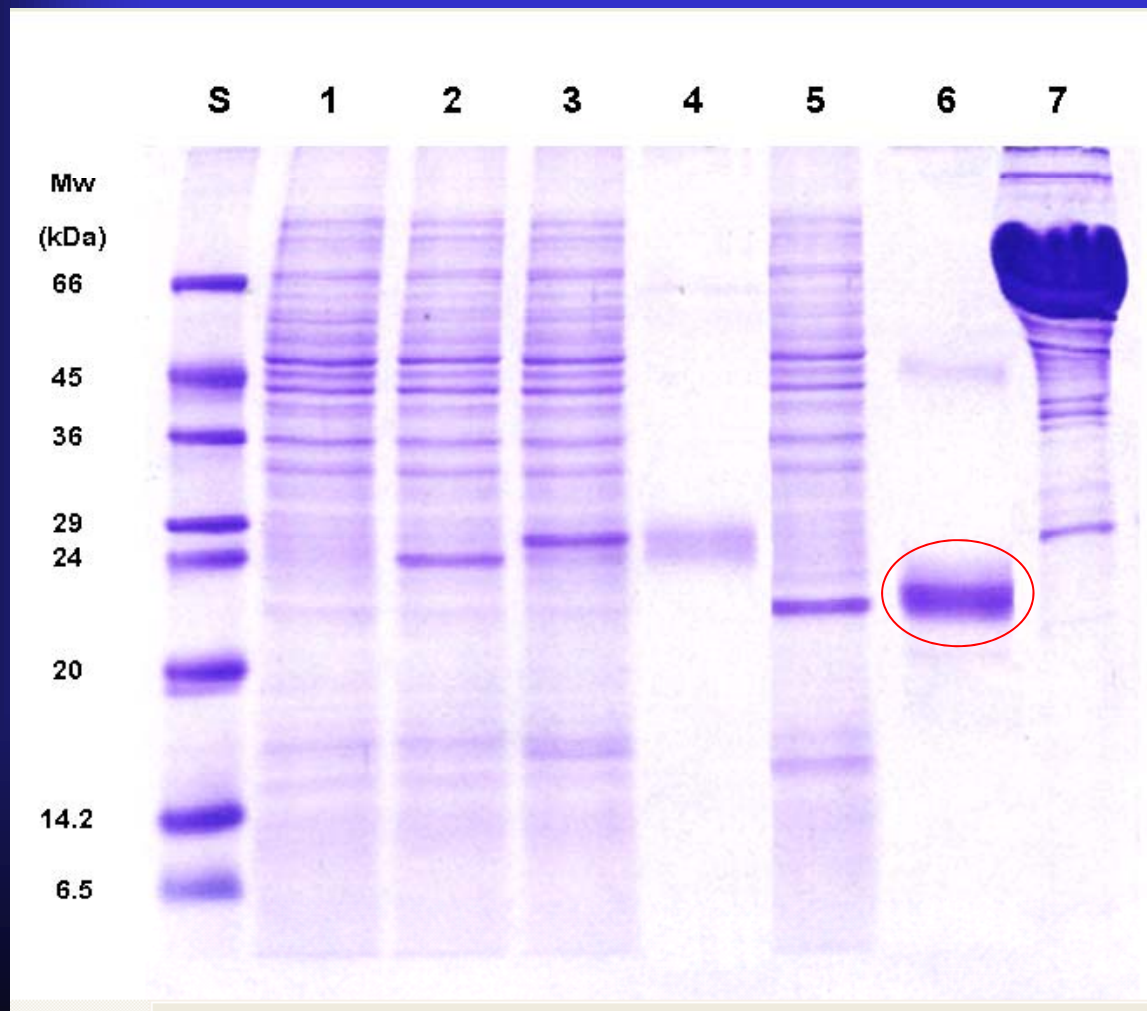
Weerawan Sithigorngul¹, Sombat Rukpratanporn², **Nilawan Pecharaburanin³**,
Siwaporn Longyant¹, Parin Chaivisuthangkura¹, Paisarn Sithigorngul^{1,*}

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²Center of Excellence for Marine Biotechnology at Chulalongkorn University, National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok 10330, Thailand

³Pacific Biotech, Petchaboon 67000, Thailand

Expression of rVP28 and rVP28F118



1 = pQE30 in M15pREP4

2 = pQE40 in M15pREP4

3 = VP28- pQE30 in M15pREP4

4 = rVP28 purified

5 = VP28F118pQE30 in M15pREP4

6 = rVP28F118 purified

7 = haemolymph of *P. monodon*
infected WSSV

Immunization

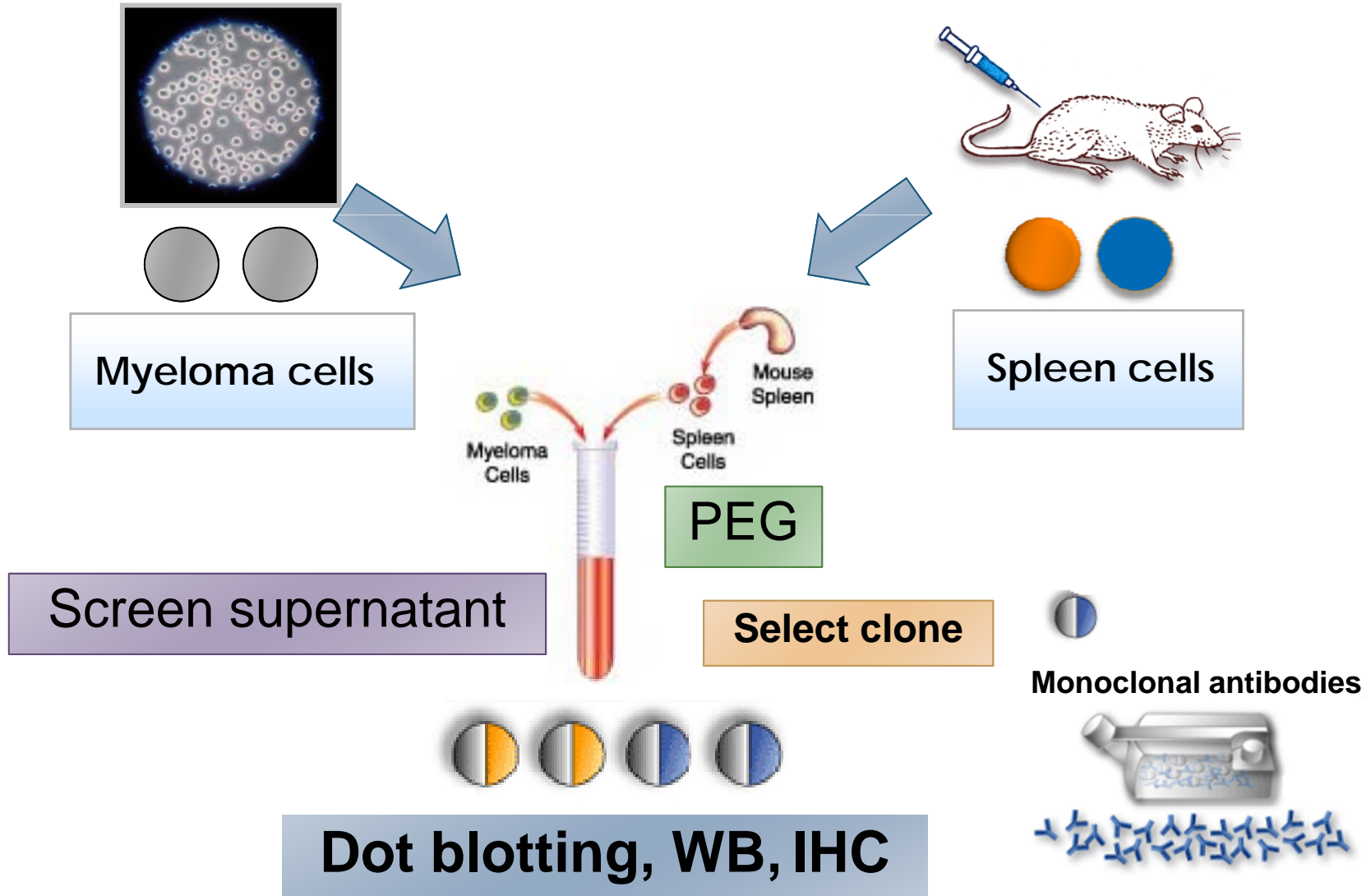
rVP28F118

+

complete Freund's adjuvant ; 1 time
incomplete Freund's adjuvant ; 3 time

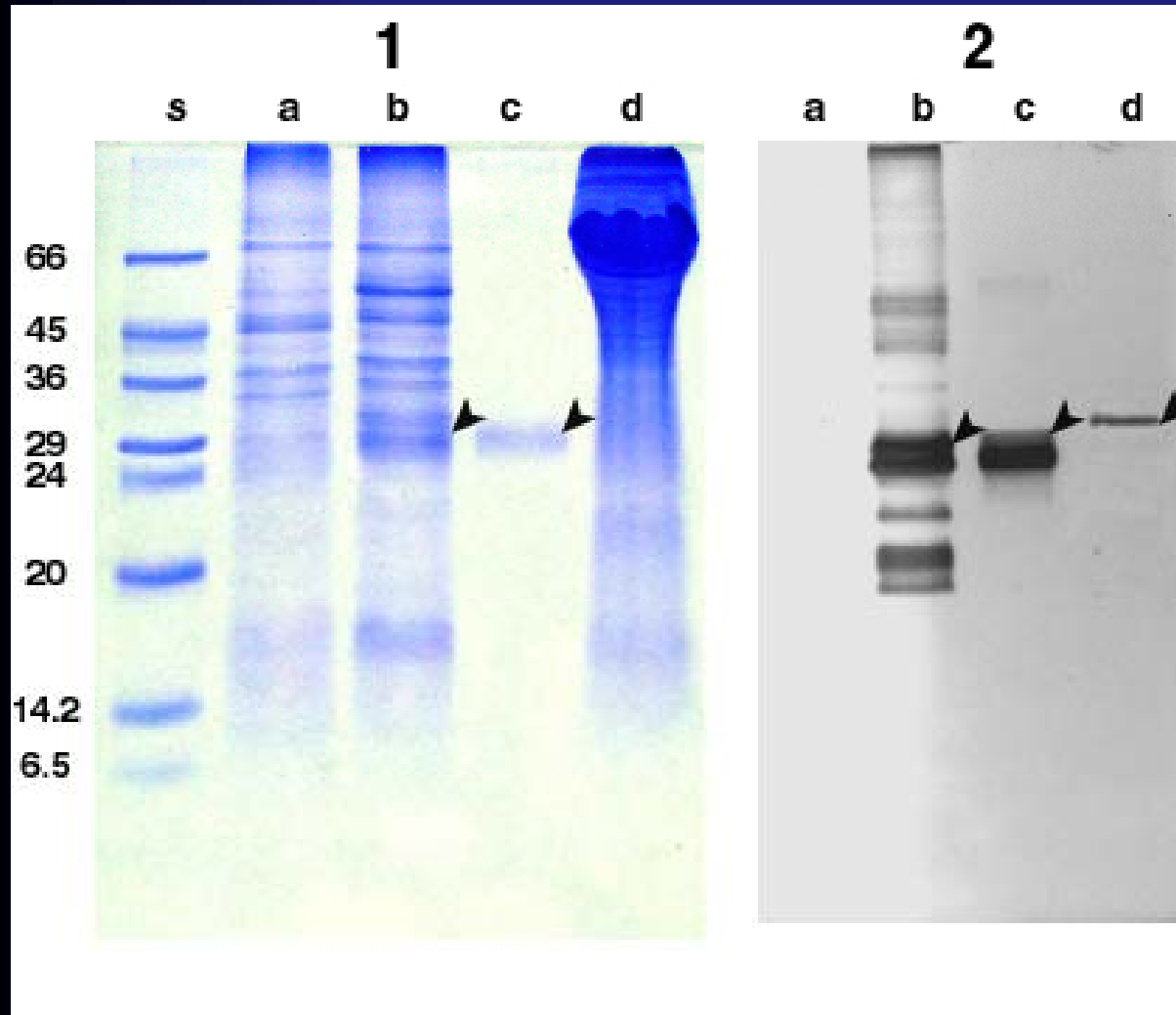


Monoclonal Antibody Production



15% SDS-PAGE and Western blotting of rVP28F118

using W29-1A monoclonal antibody



a = M15(pREP4) with pQE30

b = VP28F118-pQE30

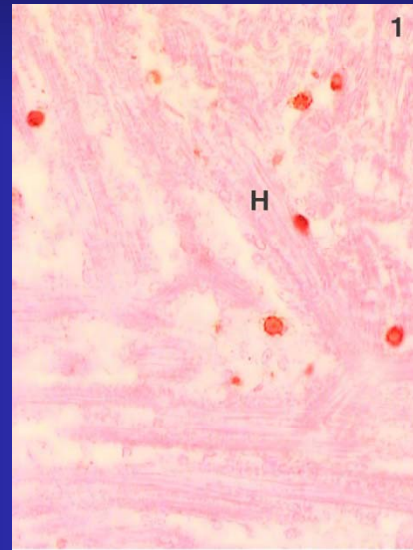
c = purified rVP28F18

d = gill homogenate from

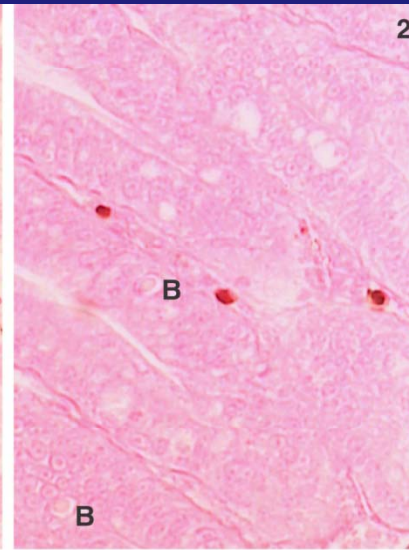
WSSV-infected *P. monodon*

Immunohistochemistry of WSSV using MAb W29-1A

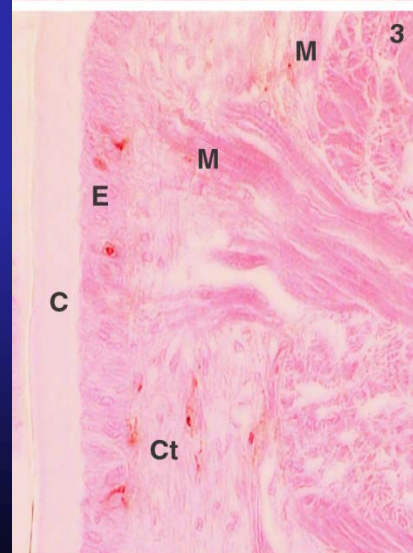
Cardiac muscle



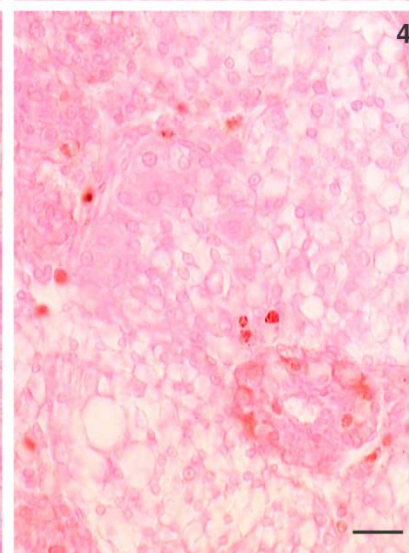
Hepatopancreas



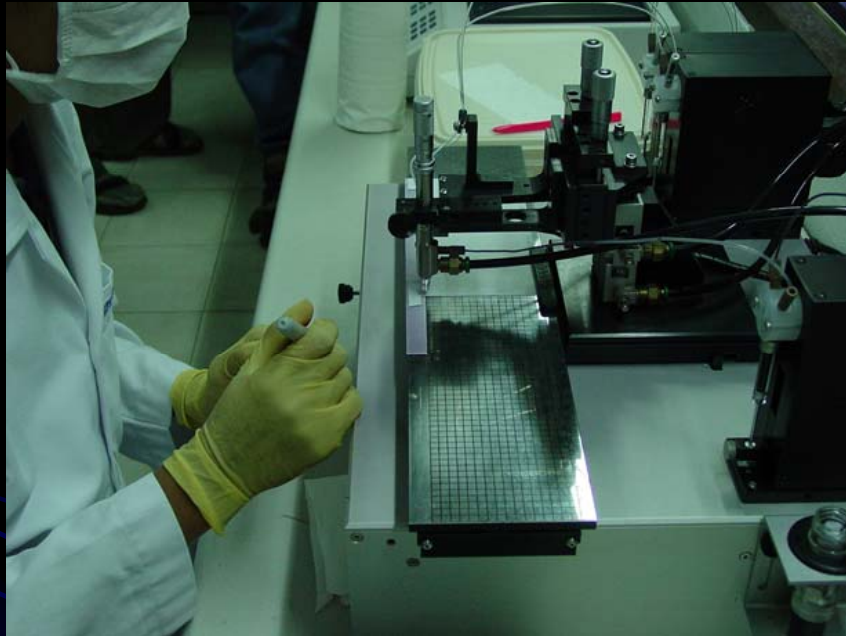
Subcuticular epithelium



Lymphoid organ



Pacific Biotech Co., Ltd.



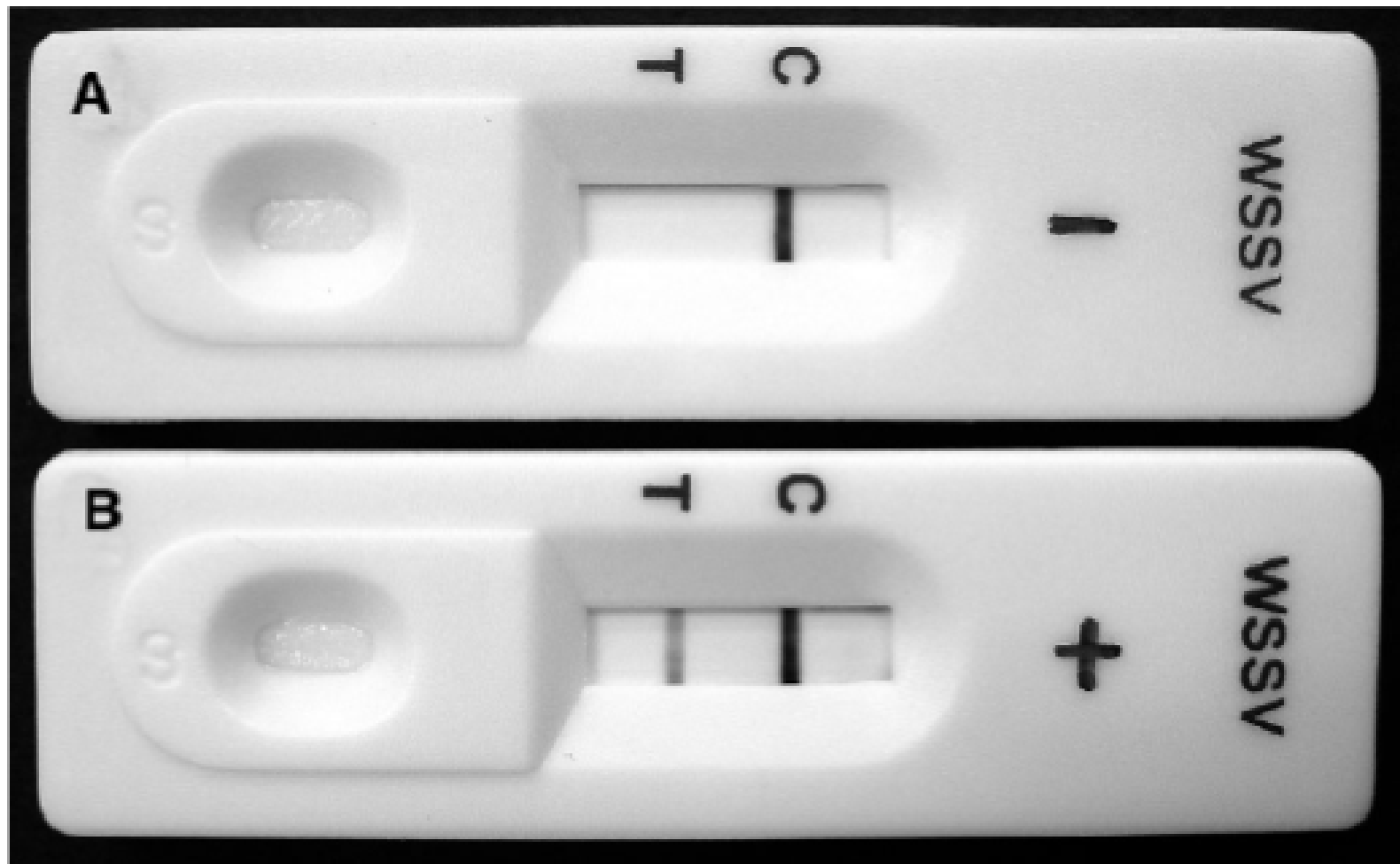
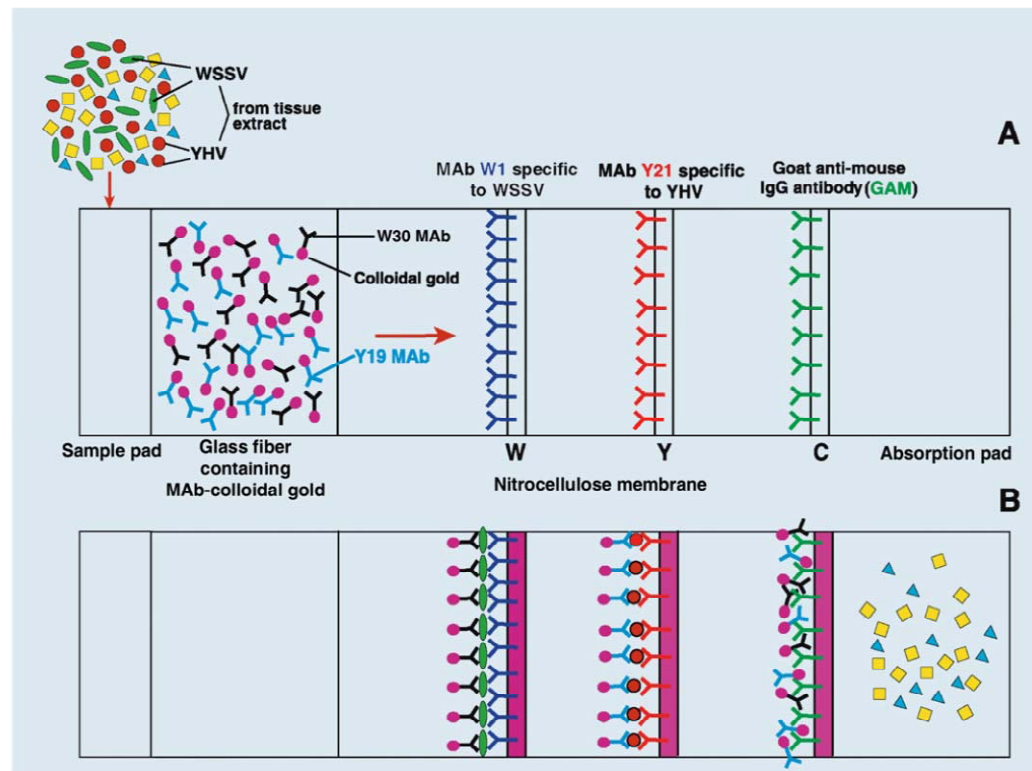


Fig. 2. Test strip. Gill homogenates from (A) uninfected and (B) WSSV-infected *Penaeus monodon* applied to the test strip.
T: test line; C: control line

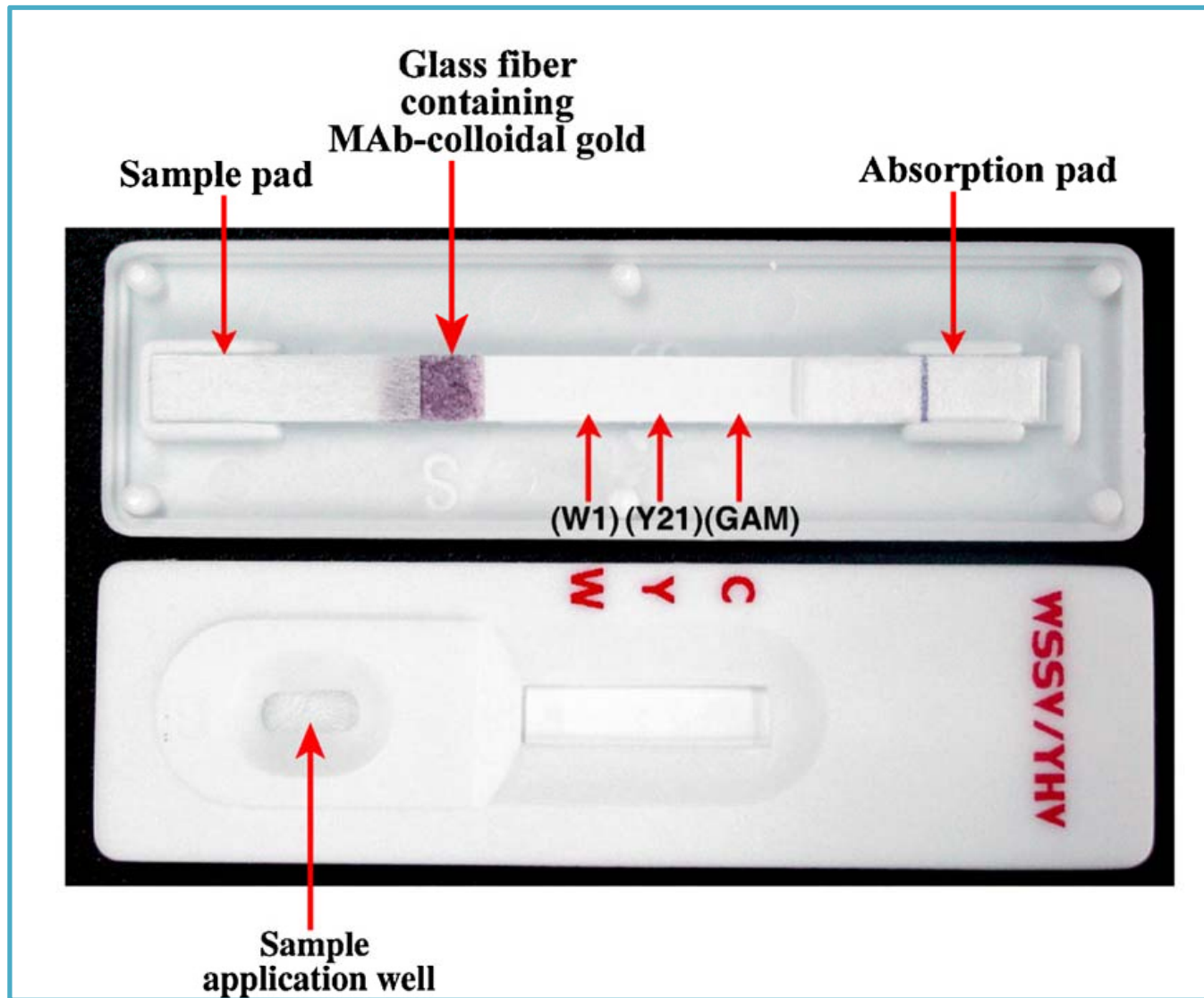


Simultaneous and rapid detection of white spot syndrome virus and yellow head virus infection in shrimp with a dual immunochromatographic strip test

Paisarn Sithigorngul^{a,*}, Sombat Rukpratanporn^b, Parin Chaivisuthangkura^a,
Pattarin Sridulyakul^a, Siwaporn Longyant^a

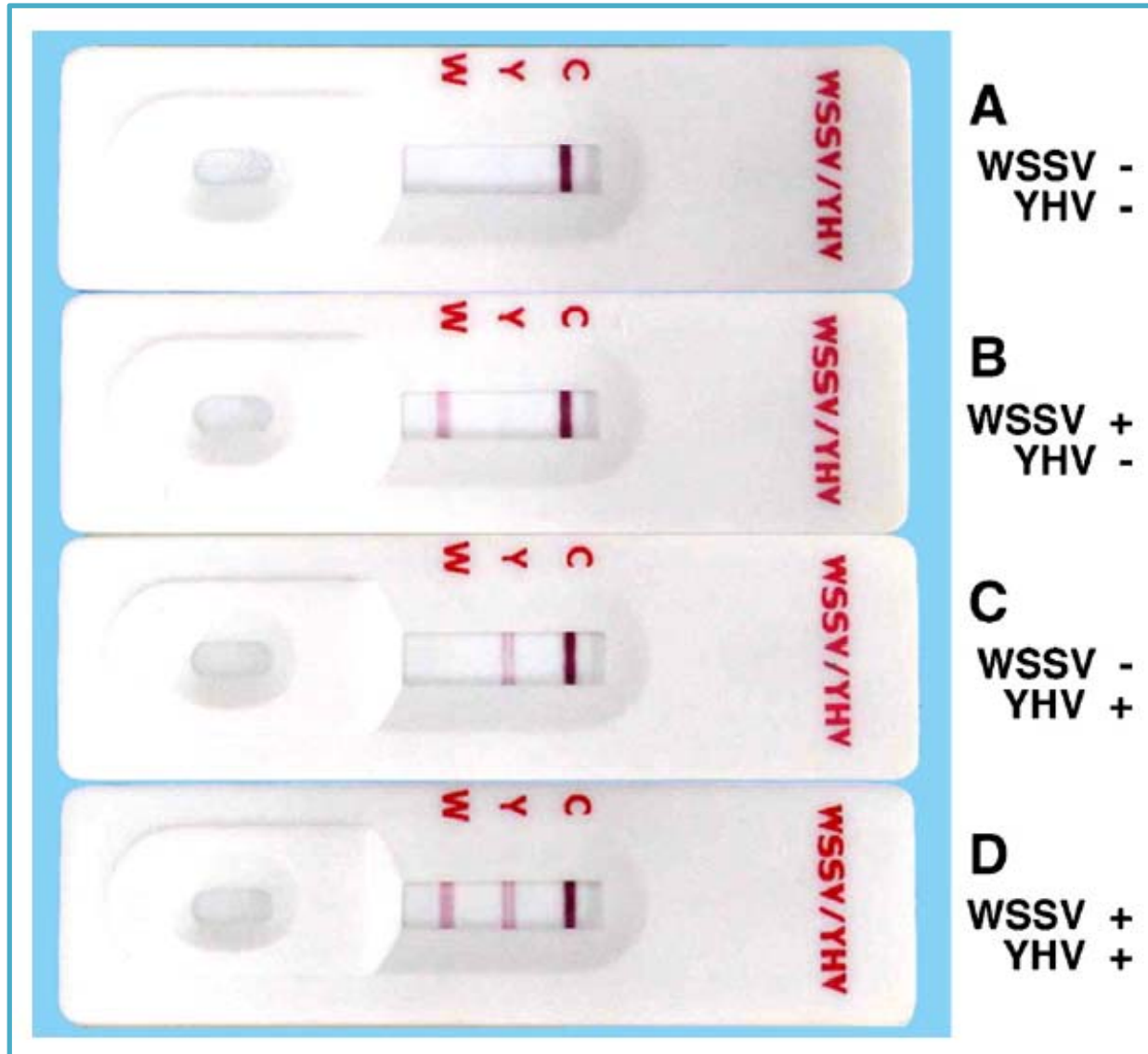


Dual Sandwich LFICS (WSSV/YHV)




Sithigorngul; et al. 2011


Dual Sandwich LFICS (WSSV/YHV)



- Distributed by Marine Leader Co. Ltd.

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MARINE LEADER

MARINE LEADER DIAGNOSIS STRIP TEST

Q-Test คิว-เทส

**DIAGNOSIS STRIP TEST
YHV+WSSV**

For Broodstock Postlarva Grow-Out Shrimp
Easy to use. Suitable for on site lab test

ชุดตรวจไวรัส YHV+WSSV

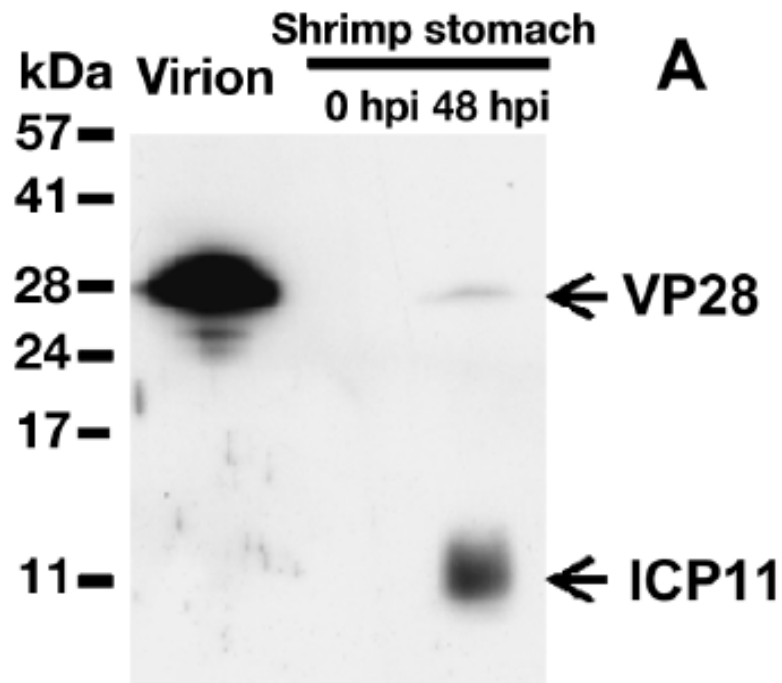
แบบแถบสี (โรคหัวเหลือง+ตัวแดงดวงขาว)



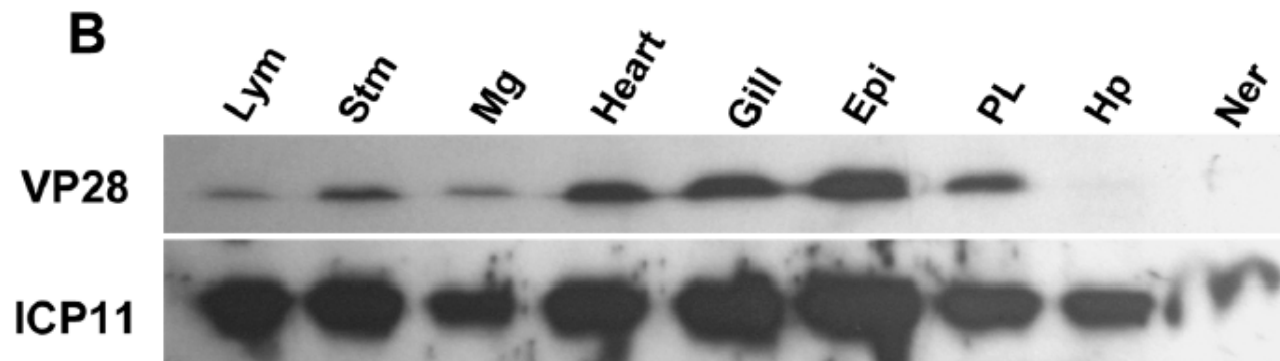
 **5 Sets**



ICP11 Protein

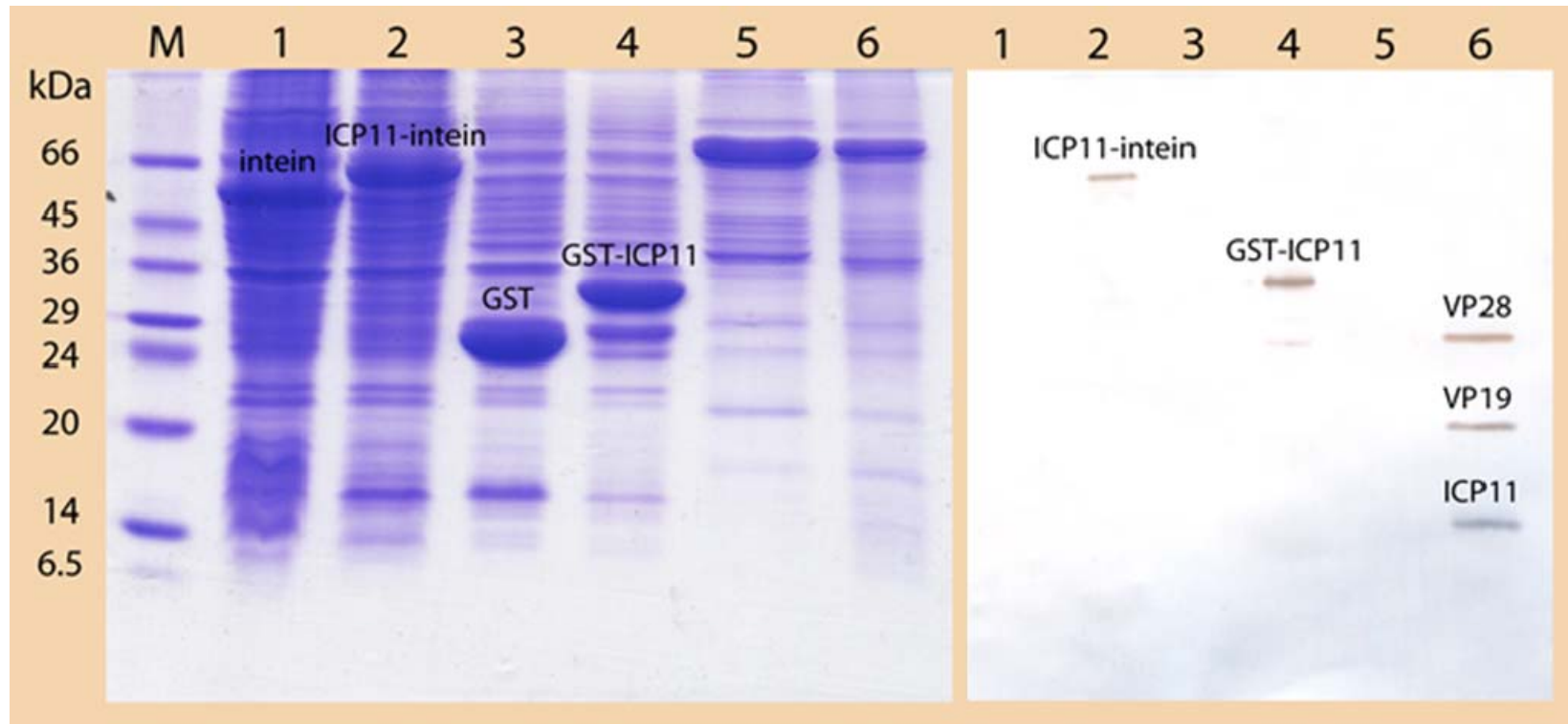


- **The most highly expressed viral gene** (3.5-fold more highly expressed than *vp28* gene).
- **ICP11, is a nonstructural protein.**
- ICP11 is a DNA mimic protein that prevents DNA from binding to histone proteins and disrupts nucleosome assembly.



Wang; et al.
(2007,2008)

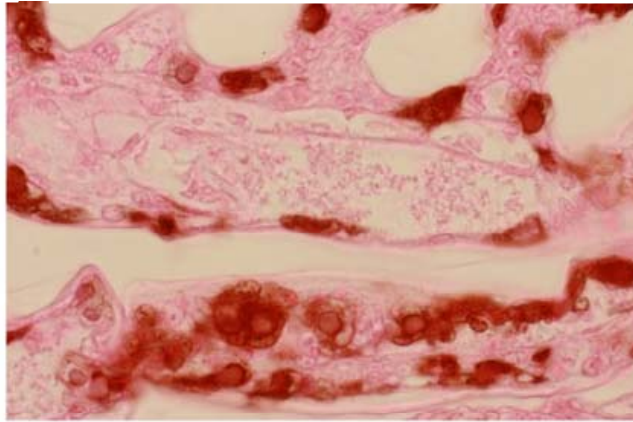
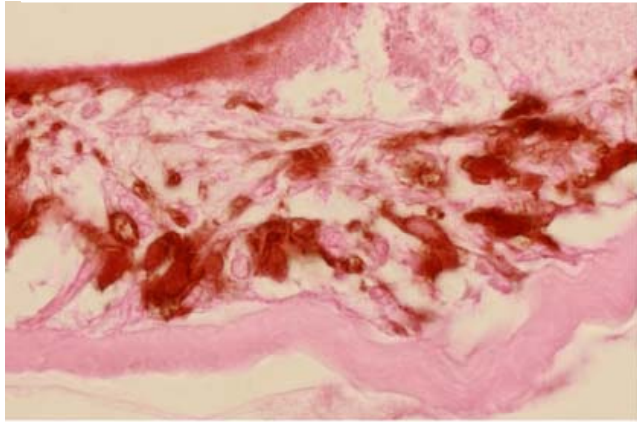
Antibody-based assays



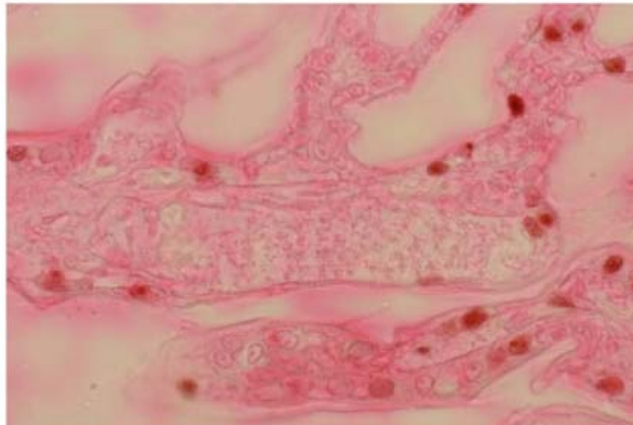
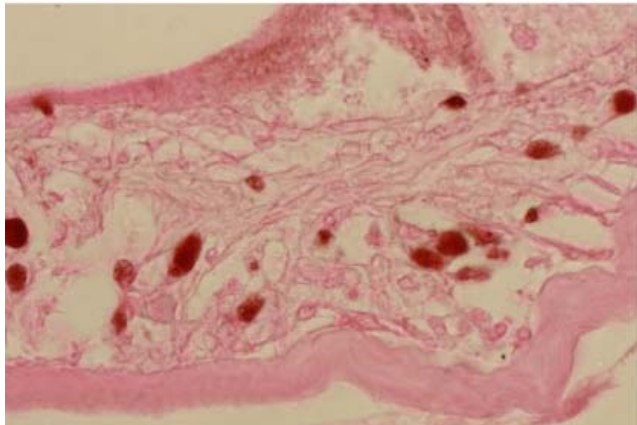
MAbs specific to ICP11 were generated using **C-terminally intein-tagged ICP11** (ICP11-intein) & **N-terminally GST-tagged ICP11** (GST-ICP11).

Subcuticular epithelium

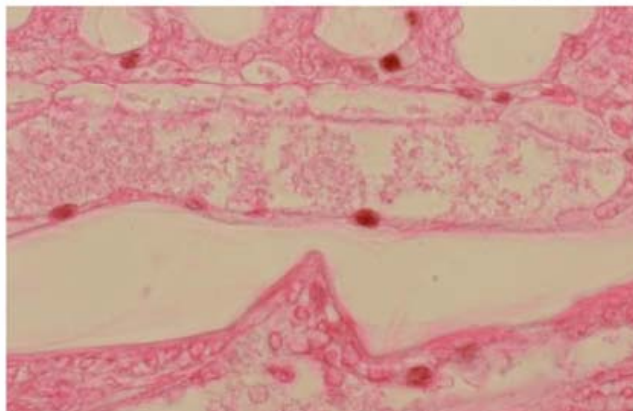
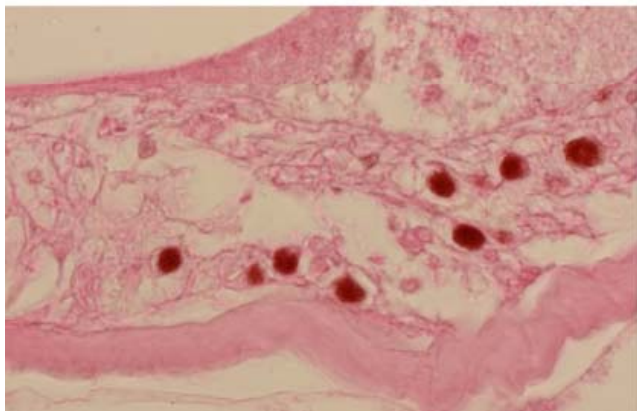
Gill



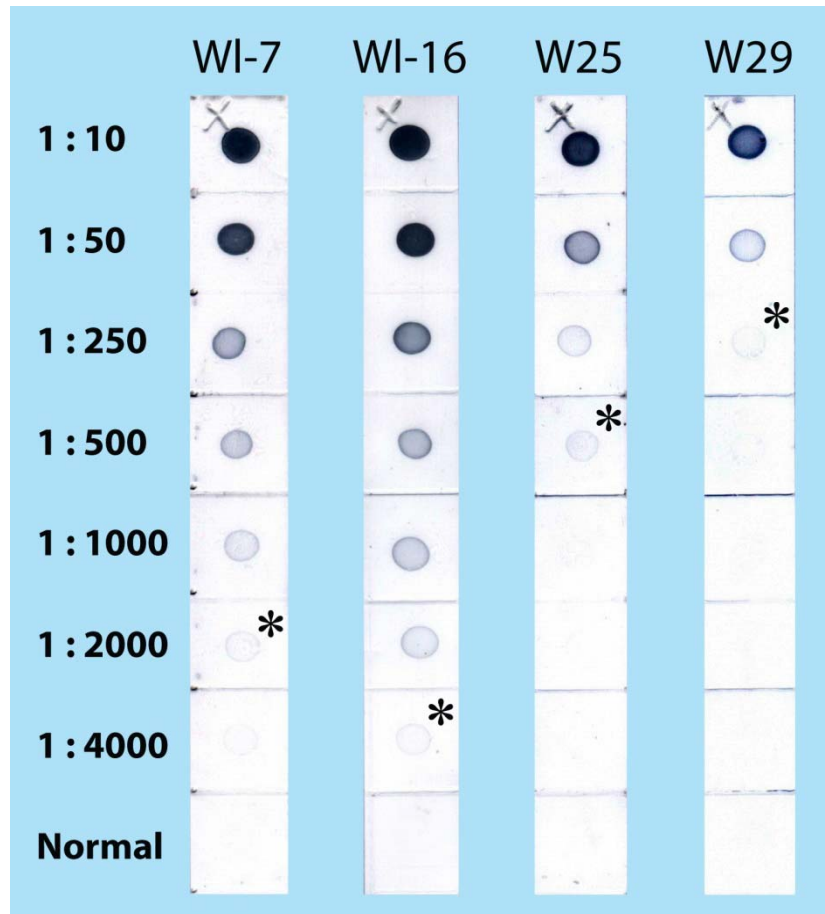
WI-16 (ICP11)



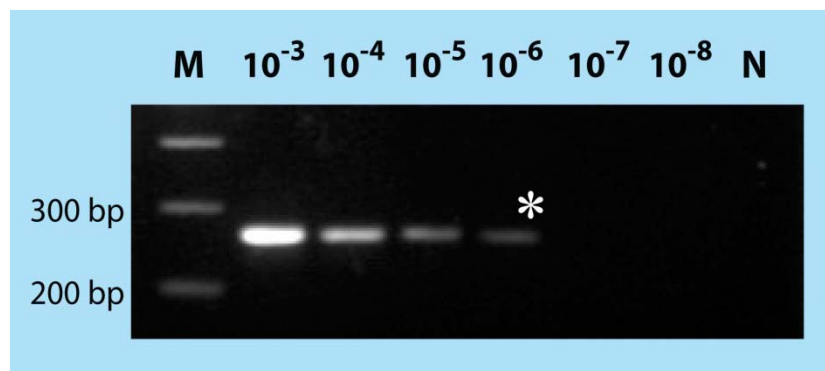
W25 (VP19)



W29 (VP28)

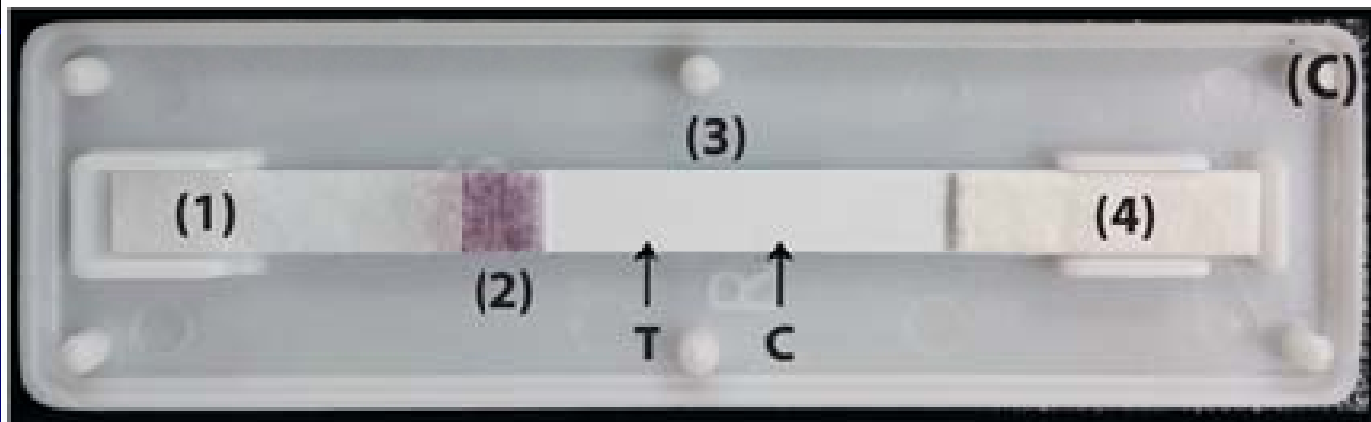
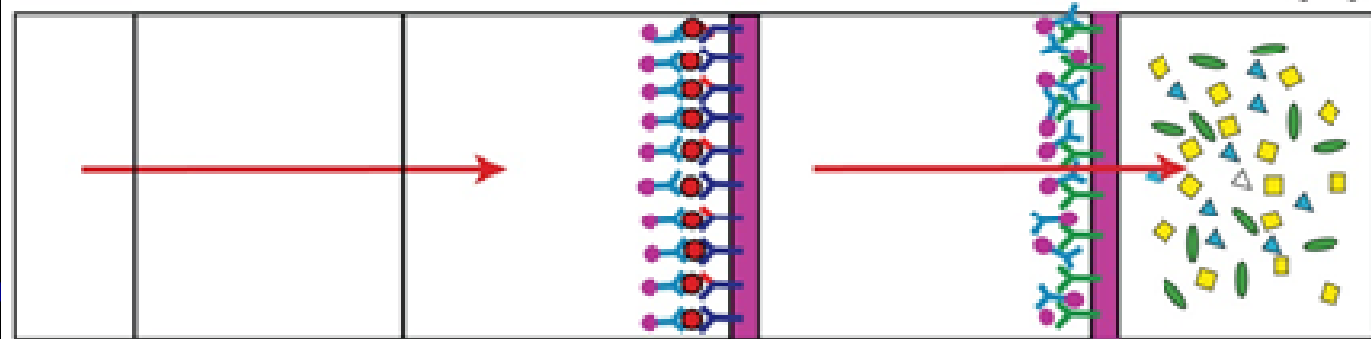
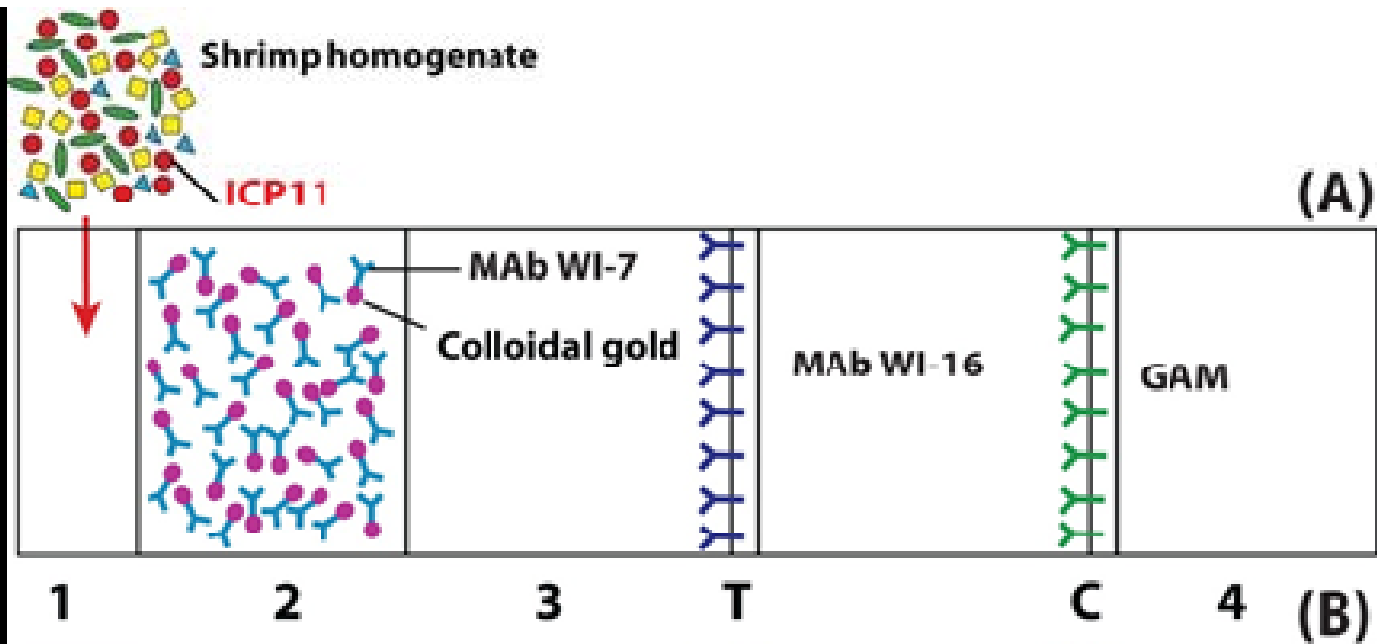


- The detection sensitivity with MAb WI-16 (ICP11) was higher than that with MAbs specific for VP19 (W25) and VP28 (W29).

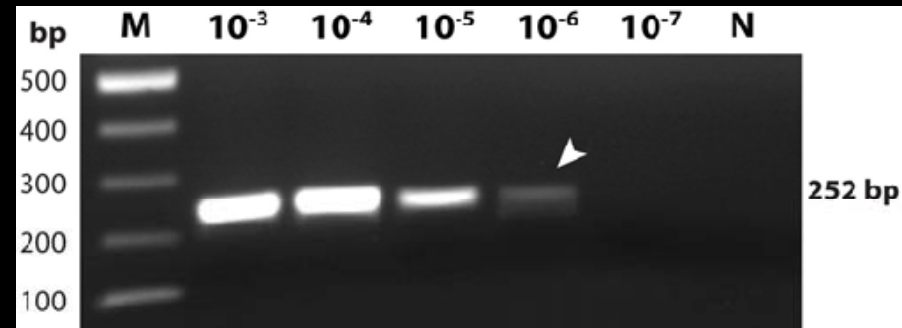
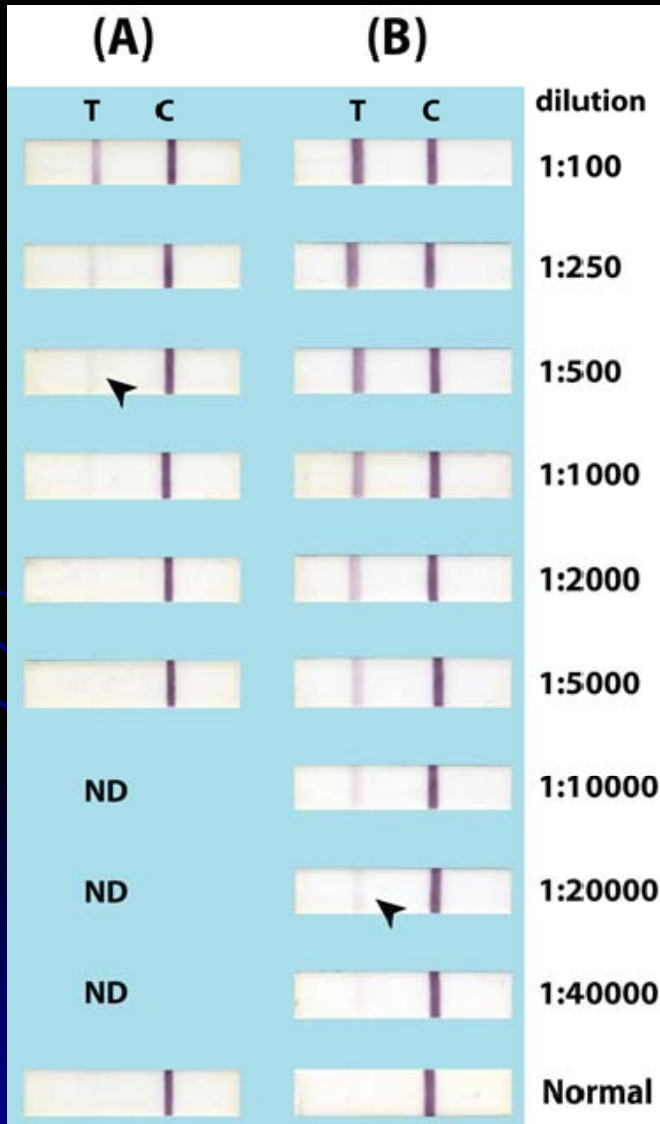


Siriwattanarat; et al.

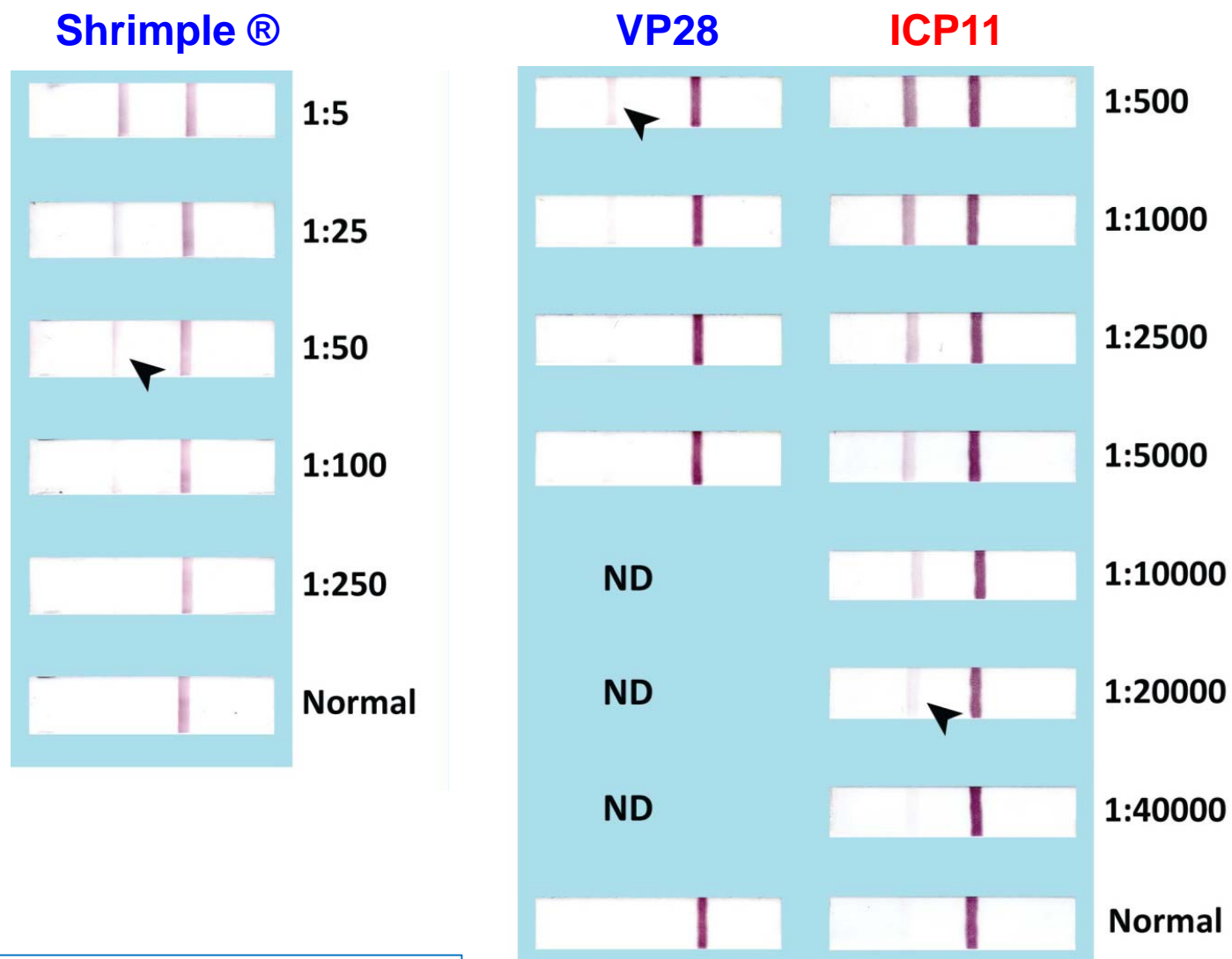
Arch Virol (2013) 158:967–979



The strip test is approximately 50 times less sensitive than one step PCR



Comparative Sensitivity



■ Sensitivity of ICP11 test strip
: 50 times less than 1-step PCR

ICP11 test strip
: 400 times more sensitive than Shrimple®



Speedy Assay Sdn Bhd 586317-W

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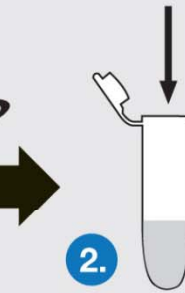
Assay Procedure (LFICS-ICP11 for WSSV)

Assay Procedure

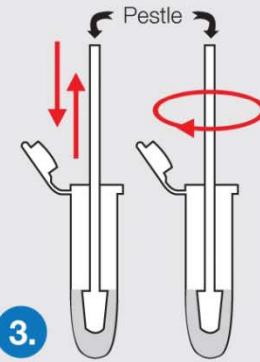
SAMPLE/SPECIMEN PREPARATION



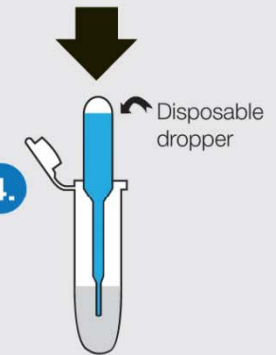
1. Cut a piece of tissue (swimmeret or walking leg)



2. Put tissue into 0.5 ml buffer (20 drops)



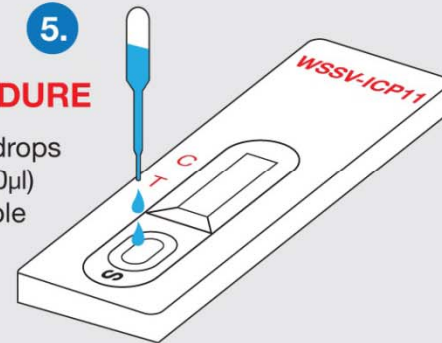
3. Grind sample into small pieces



4. Draw the sample

ASSAY PROCEDURE

5. Add 5-6 drops (100 - 150 μ l) into sample chamber



6.



Read result in 15 minutes

Ready-to-use Strip test



Marketing Strip tests by Profound Kestrel Lab.


Ordering Information

Catalogue No.	Description	Pack Size
RWSS01-020	White Spot Syndrome Virus (WSSV) Rapid Test Kit	20 tests/ kit

Other related products

Catalogue No.	Description	Pack Size
RIMN01-020	Infectious Myonecrosis Virus (IMNV) Rapid Test Kit	20 tests/ kit
RYHV01-020	Yellow Head Virus (YHV) Rapid Test Kit	20 tests/ kit
RMBV01-020	Monodon Baculovirus (MBV) Rapid Test Kit	20 tests/ kit

Marketing in Thailand: Marine Leader Co. Ltd.



Q-TEST

White Spot Syndrome Virus

วิธีอ่านผล
Reading the result

WSSV Positive
พบเชื้อไวรัส WSSV

any viruses Negative
ไม่พบเชื้อ

The red/purple bands on the W and control (C) lines indicate WSSV and infection respectively. If a band only occurs at the C line, indicates the negative result or the infection is too low to be detected by the test kit.

BENEFITS

- Easy to use: The method is must simple and unskilled person can do at pond site without any additional equipment.
- Low cost: Testing cost lower than that of PCR
- Virus-spread control: Real-time result help to stop virus epidemics immediately

APPLICATION

Detection of WSSV in shrimp tissue within 15-30 min

CAUTIONS

- This test kit is for primary screening or confirmation of the WSSV infection. The negative result should be confirmed by PCR or RT-PCR or immunohistochemistry.
- The light gray line may be observed at the W line while the strip is still wet. It indicates the deposition of haemocyanin. It is still considered as negative result.
- Gill and lower part of the swimmeret is the best source since they consist of large area of epithelium, the target of WSSV infection. The amount of tissues should increase if smaller size of shrimp was used.
- To prevent contamination of the virus, all materials contacted with the sample should be treated with dilute bleach (hypochlorite) solution before discard or reuse.


SPECIFICATIONS:

Shelf-life : 1 year from date of manufacture

Storage : Product should be kept in its original package and stored in a cool, dry location away from direct sunlight.


Packaging : 5 sets/ box

วิธีการอ่าน / Test Procedure



1. สับ A หรือ B หรือ C 1 ชิ้น นำมาบดละเอียดใส่หลอดปั่น
2. สุกไฟน้ำใน 0.15-0.2 น. สกัดและกรองด้วยกระดาษ 0.6 ไมครอน แล้วล้างใน 15-30 นาที

1. Squeeze the tissue sample such as 1 strip of gill, 1 walking leg or 1 swimmeret (from shrimp with 15-20 gram body weight) in 0.3 ml buffer provided in the microcentrifuge with pestle.
2. Withrow 0.15-0.2 ml (5-8 drops) of the supernatant and slowly apply into the sample well, read the result at 15-30 min.



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CONTACT INFORMATION
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Probiotic

Chemicals

Water Quality Test Kit

Raw material and feed additive

Nursery feed or Race way feed

Products : **Diagnostic Strip Test**



Code : **J0001**

Name : **Q – Test (WSSV/YHV Strip Test)**

Q – Test WSSV Strip Test
Detection of WSSV in shrimp tissue within 15 min



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Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Sensitivity improvement of immunochromatographic strip test for infectious myonecrosis virus detection



Pradit Wangman ^a, Siwaporn Longyant ^a, Heny Budi Utari ^b, Saengchan Senapin ^{c,d}, Chalinan Pengsuk ^e, Paisarn Sithigorngul ^a, Parin Chaivisuthangkura ^{a,*}

^a Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand

^b Charoen Pokphand Group of Indonesia, Bogor, West Java, Indonesia

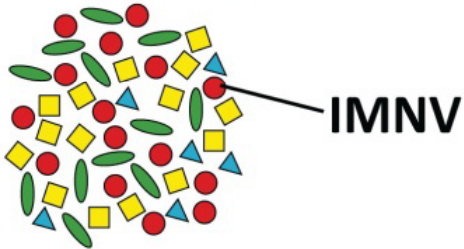
^c Center of Excellence for Shrimp Molecular Biology and Biotechnology, Mahidol University, Bangkok 10400, Thailand

^d National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathum Thani 12120, Thailand

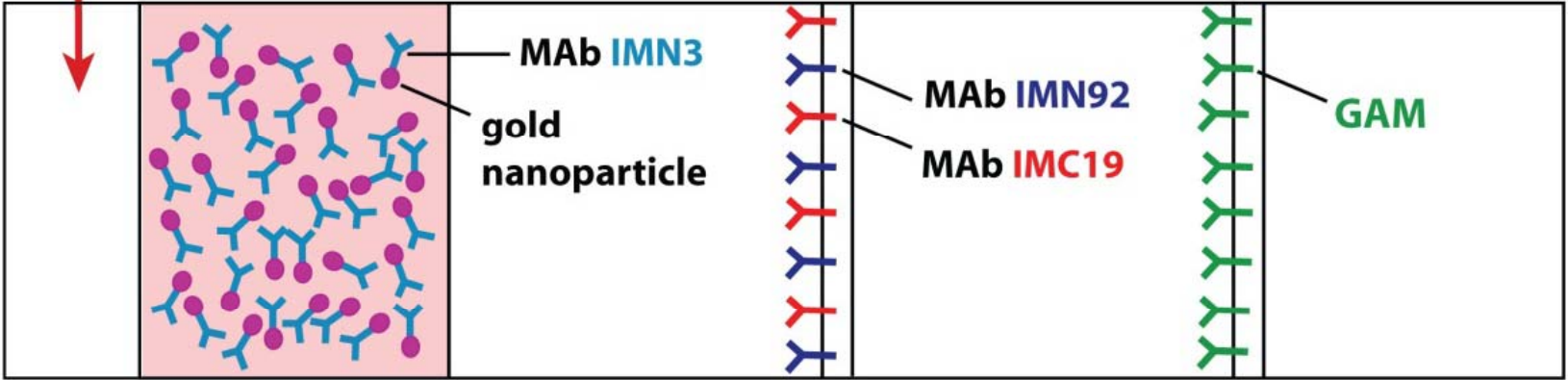
^e Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, Bangkok 10110, Thailand

IMNV Sandwich LFICS

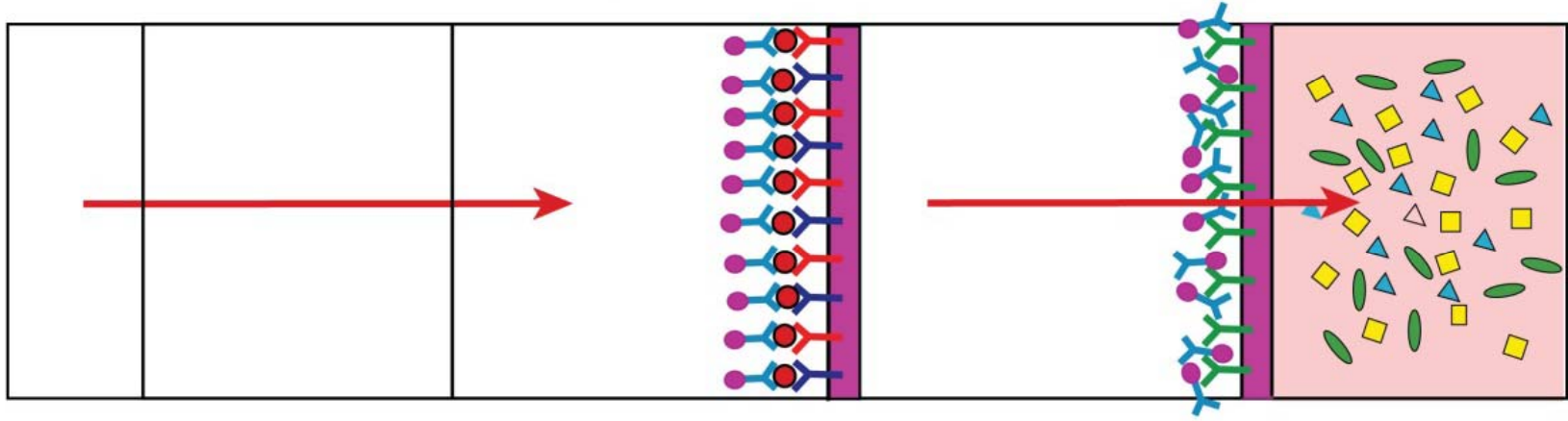
Shrimp homogenate



A



B



IMNV Sandwich LFICS



A B S T R A C T

An immunochromatographic strip test with enhanced sensitivity for the detection of infectious myonecrosis virus (IMNV) was developed using three monoclonal antibodies specific to N- and C-termini fragments of IMNV capsid protein. This strip test can detect IMNV with 20 times higher sensitivity than the previous test strip and approximately 50 times lower than that of one-step RT-PCR. The shrimp sample can be heat-treated to augment the release of antigen from the muscular tissue and ensure the sterilization of the sample. Due to its high specificity, field friendly and rapid result, this test strip is suitable for surveillance of IMNV outbreaks and confirmation of IMNV infection in shrimp farming.

Statement of relevance: The IMNV strip test will help farmers to monitor IMNV infection during shrimp culture.

The test kit has sensitivity of approximately 50 times lower than that of one-step RT-PCR.

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Development and evaluation of a highly sensitive immunochromatographic strip test using gold nanoparticle for direct detection of *Vibrio cholerae* O139 in seafood samples



Chalinan Pengsuk, Parin Chaivisuthangkura, Siwaporn Longyant, Paisarn Sithigorngul*

Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand

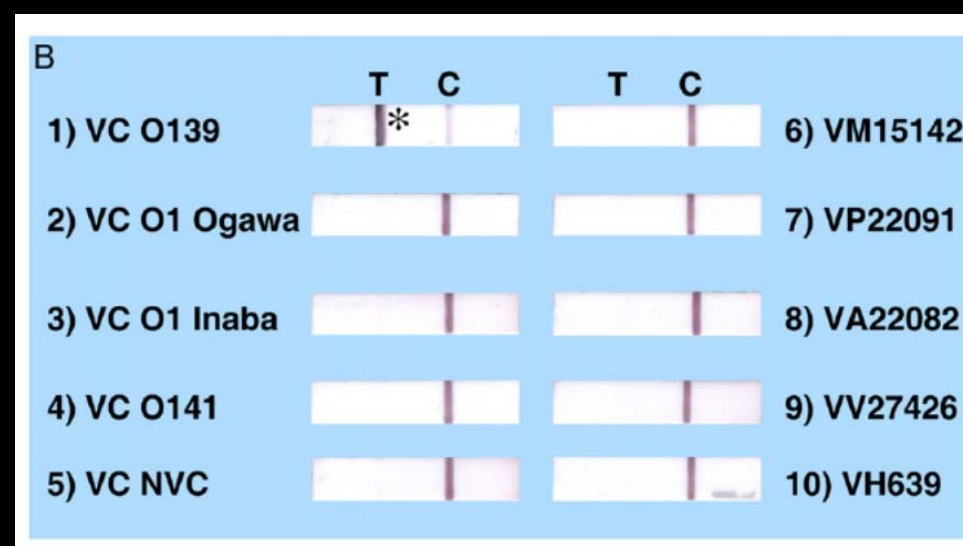
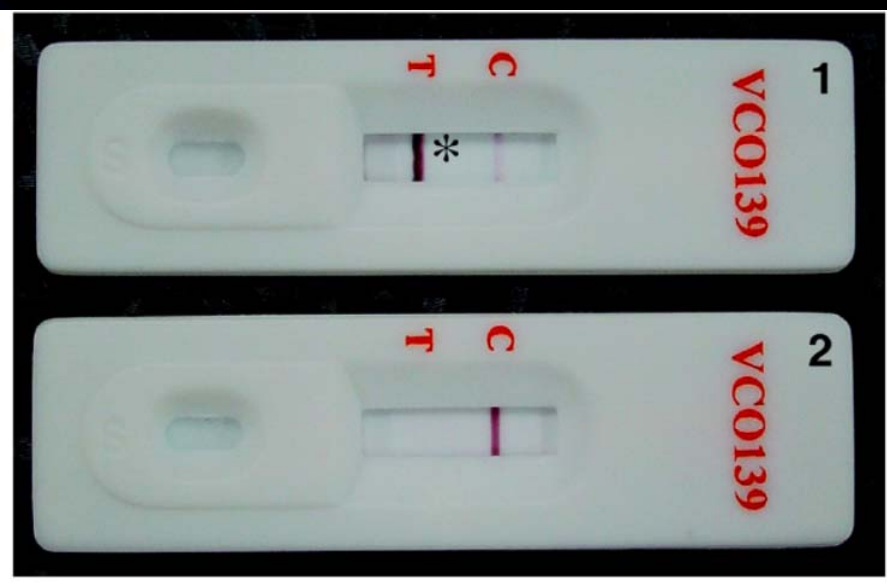




Fig. 5. Detection sensitivity of the strip test after pre-enrichment in shrimp homogenate in APW. A similar experiment was performed using shrimp homogenate in APW (1:10=weight:volume) as diluents. * represents the lowest detection limit.

Sensitivity of VC O139 in spiked shrimp

- 10^4 CFU/ml without enrichment

• - 10^2 CFU/ml after incubation in APW for 6 hr.

Virus	Specificity	Immuno assays	Strip Test	References
	VP19	+	-	Chaivisuthangkura et al. 2010
WSSV	VP26	+	-	Vaniksampanna et al. 2016
	VP28	+	+	Sithigorngul et al. 2012
	ICP11	+	+	Siriwattanarat et al. 2012
YHV	nucleocapsid	+	+	Sithigorngul et al. 2012
MBV	polyhedrin	+	+	Wangman et al. 2011
IMNV	CP	+	+	Wangman et al. 2016
HPV	CP	+	-	Srisuk et al. 2011
IHHNV	CP	+	-	Sithigorngul et al. 2012
	VP1	+	-	Hajimasalaeh et al. 2013
TSV	VP2	+	-	Chaivisuthangkura et al. 2010
	VP3	+	-	Longyant et al. 2008
MrNV	CP	+	-	Wangman et al. 2012
XSV	CP	+	-	Longyant et al. 2012



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Development of monoclonal antibodies specific to ToxA and ToxB of *Vibrio parahaemolyticus* that cause acute hepatopancreatic necrosis disease (AHPND)



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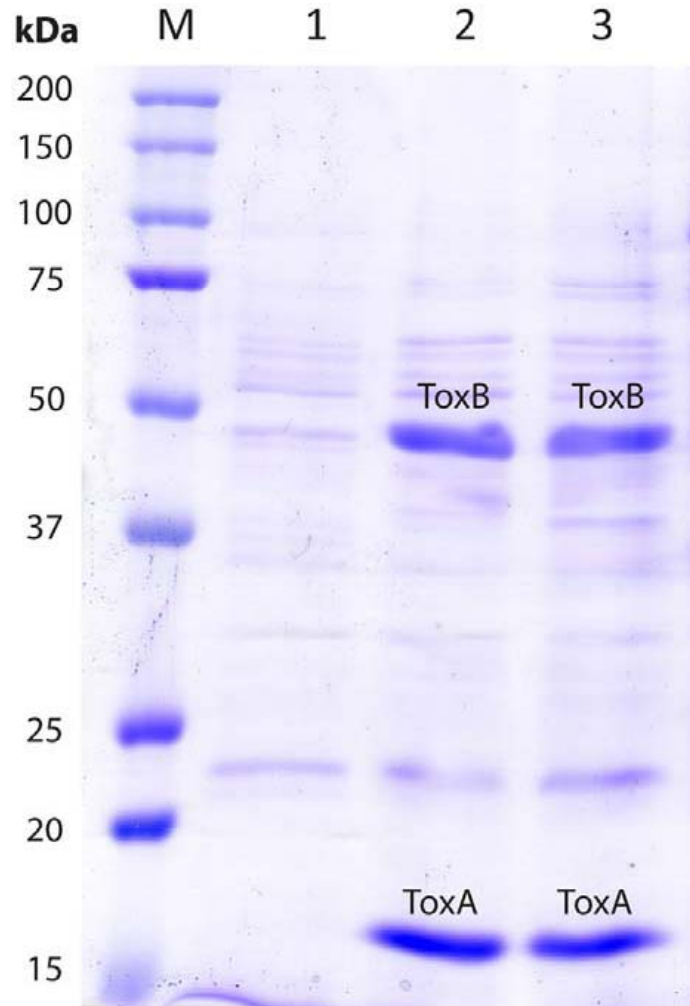
^c Shrimp-Pathogen Interaction (SPI) Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Yothi Office, Bangkok 10400, Thailand

^d Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Mahidol University, Bangkok 10400, Thailand

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^f Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, Bangkok 10110, Thailand

Preparation of ToxA and ToxB supernatant for immunization



SDS-PAGE protein profiles of supernatants from :

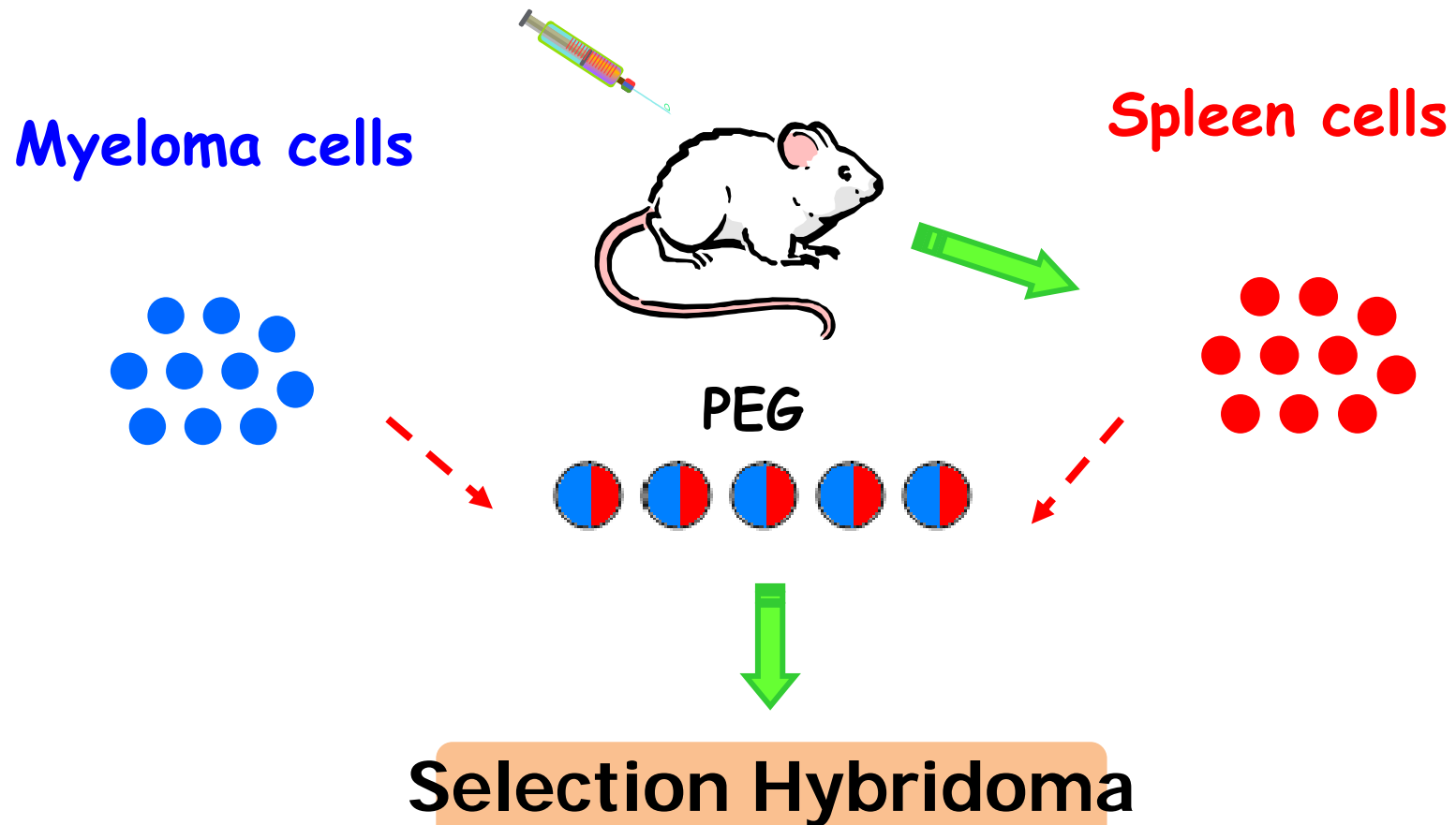
(1) non-VP_{AHPND}

(2) CN-VP_{AHPND}

(3) 5HP-VP_{AHPND}

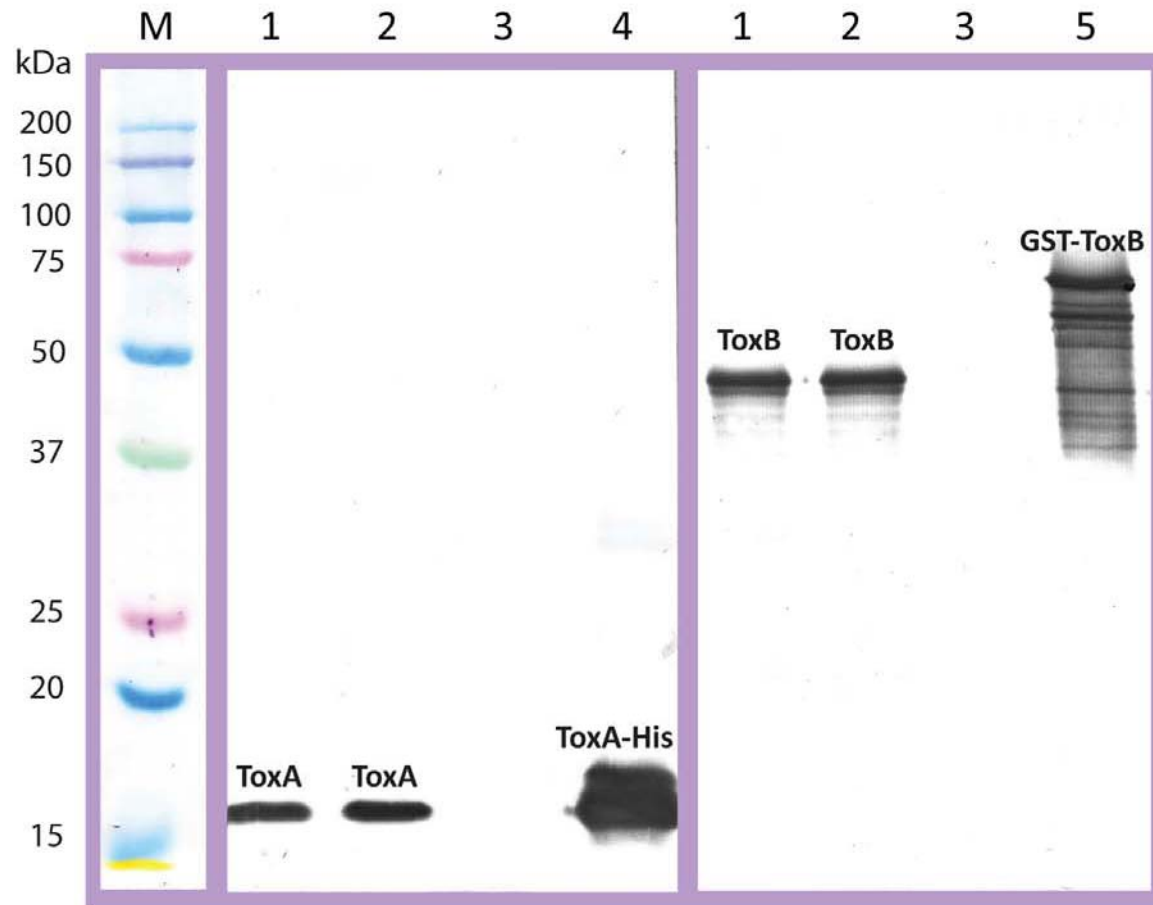
Monoclonal Antibody Production

Supernatant (ToxA & ToxB) from CN-VP_{AHPND}



MAb: A-14E

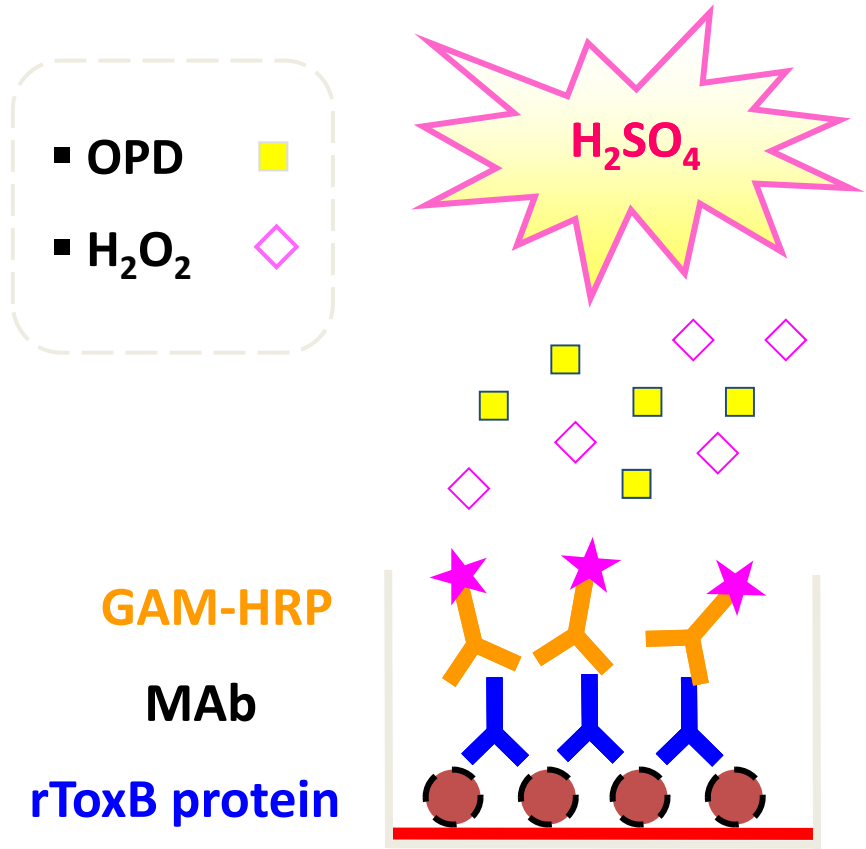
MAb: B-11E



- (1) 5HP-VP_{AHPND}
- (2) CN-VP_{AHPND}
- (3) non-VP_{AHPND}
- (4) ToxA-His
- (5) GST-ToxB

MAbs	Sensitivity dot blotting	western blotting	Specificity
A-14E (IgG2b)	200 fmole/ul	+	ToxA
B-11E (IgG2b)	10 fmole/ul	+	ToxB
B-12C (IgG1)	10 fmole/ul	+	ToxB
B-13D (IgG2b)	20 fmole/ul	+	ToxB

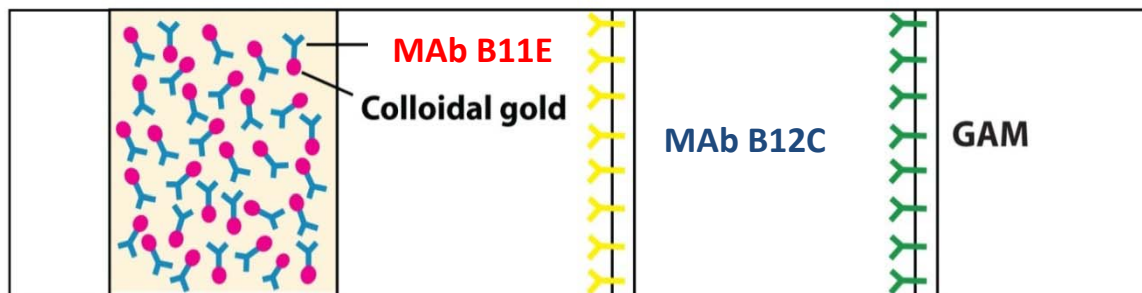
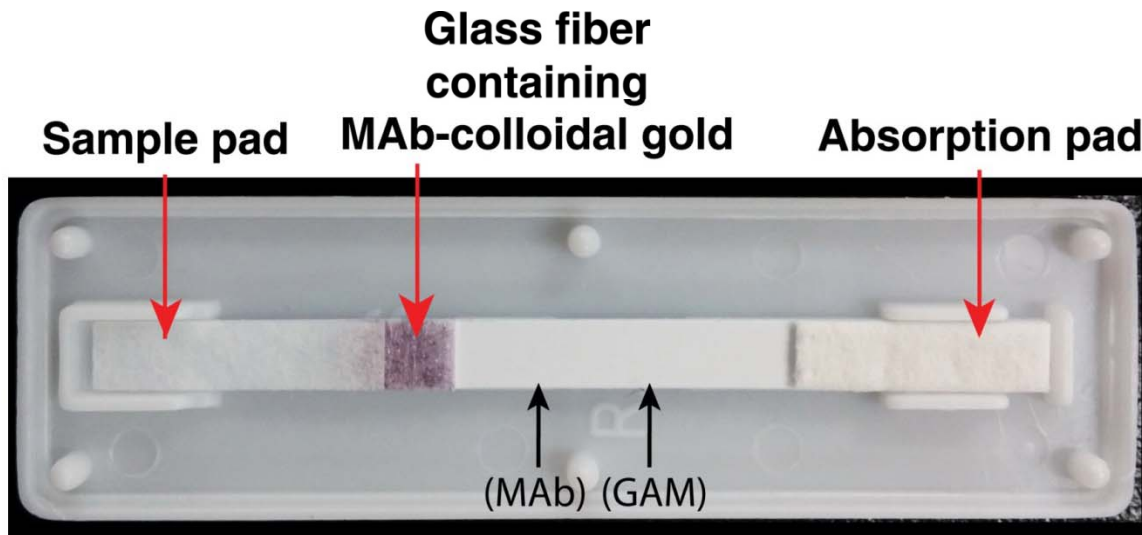
Checker board Indirect ELISA



MAbs	B-12C	B-13D	B-11E
B-12C	0.493 ¹	0.475	0.699
B-13D		0.424 ¹	0.623
B-11E			0.525 ²

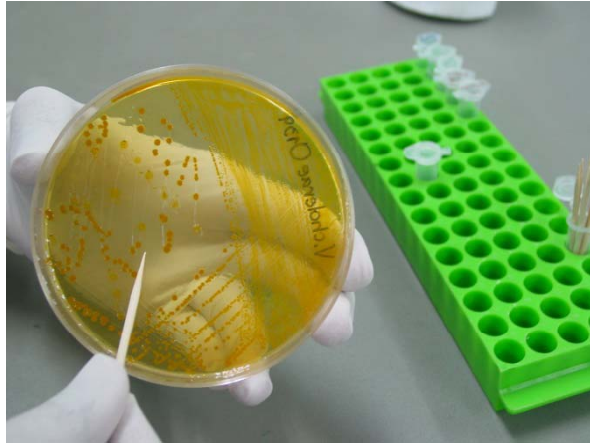
**Coming Soon
Strip Test for EMS!**

Preparation of EMS (ToxB) strip test



- Mabs were purified by protein A or G column.
- **MAb B-11E** was conjugated to colloidal gold & sprayed onto a glass fiber pad.
- **Mabs B-12C** was sprayed onto nitrocellulose mb. at test line
- **GAM** was sprayed onto nitrocellulose mb. at control line

EMS Strip Test



Acknowledgments



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**Dr. Saengchan
Senapin**



**Dr. Kallaya
Dangtip**



Dr. Chalinan Pengsuk



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