Welcome to NFQS

New OIE reference laboratory for Viral Haemorrhagic Septicaemia (VHS) in Korea

Hyoung Jun Kim

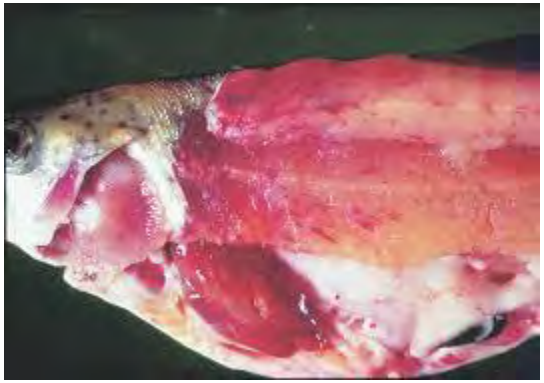
National Fishery Products Quality Management Service, Korea
A newly designated expert of OIE reference laboratory for VHS

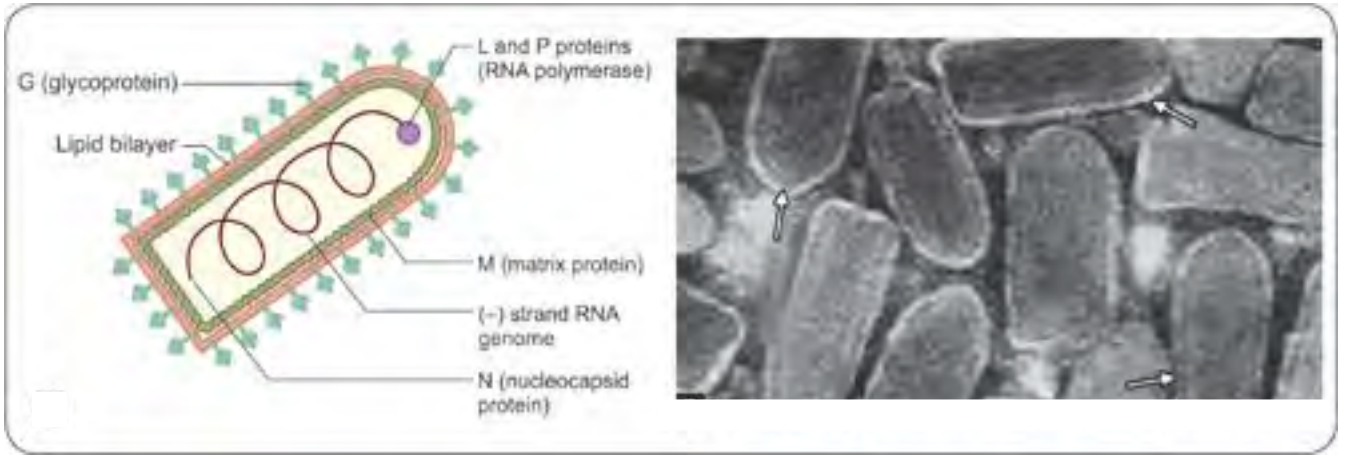
해양수산부
국립수산물품질관리원

OIE REFERENCE LABORATORY
VIRAL HAEMORRHAGIC SEPTICAEMIA

Background

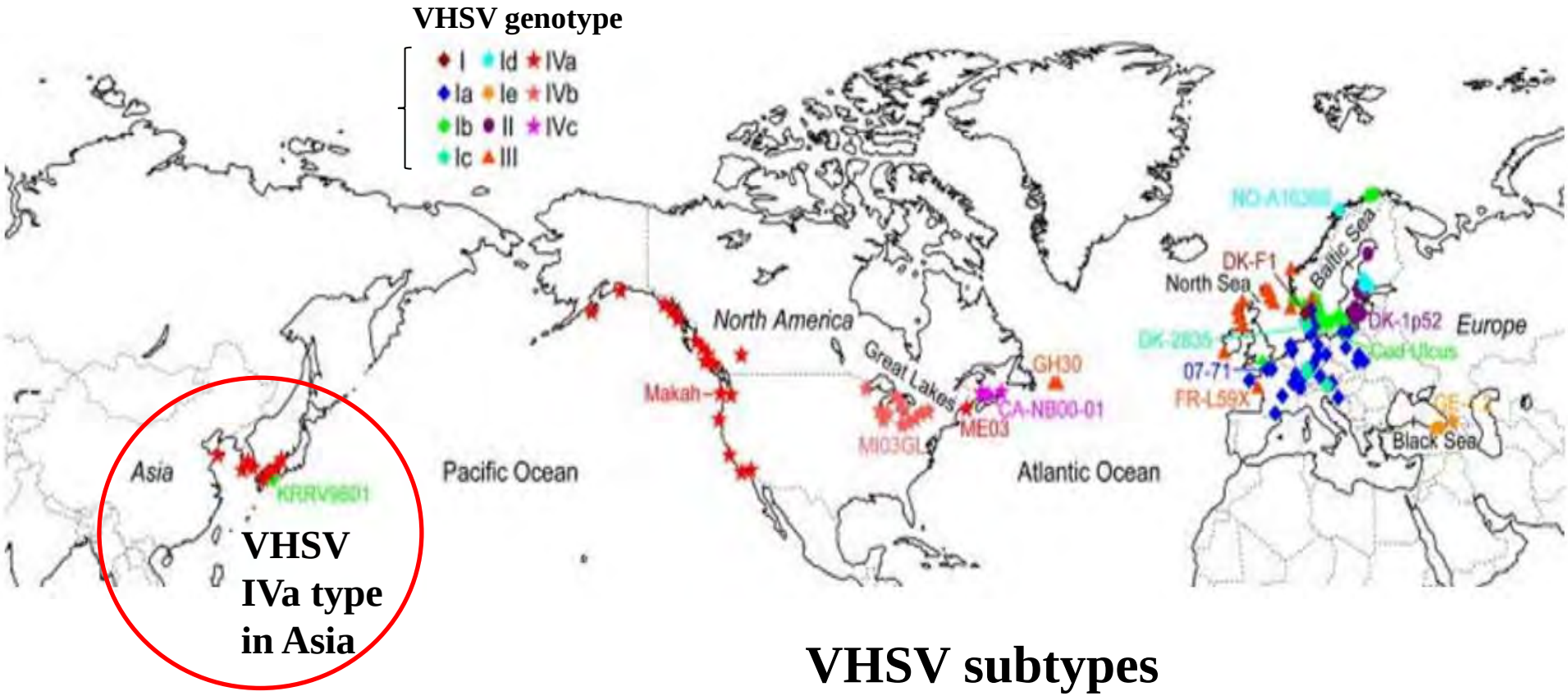
Viral Haemorrhagic Septicaemia (VHS) ?





Background

Global distribution of VHSV (Genotype I, II, III, IV)



VHSV IVa type in Asia

VHSV subtypes

I : Ia, Ib, Ic, Id, Ie **II**

III : IIIa, IIIb **IV : IVa, IVb, IVc, IVd**

Background


발 간 등 록 번 호
11-1192073-000004-01
www.nfqs.go.kr
국립수산물품질관리원 중장기 비전
New Start 2020
2013
FIQ 국립수산물품질관리원

Background

Invitation of OIE reference laboratory experts to NFQS in 2014

Invitation of OIE reference laboratory specialists in NFQS

Name	Organization	Signature
HNIN THIDAR MYINT	OIE TOKYO	
NIELS JØRGEN OLESEN	DTU-VETERINARY	
A.S. SAHUL HAMEED	OIE, INDIA CABL	
Chu-Fang Lo	NCKU Tainan Taiwan	
Pen-Hsiang CHANG	National Taiwan University	
Hirofumi Kugita	OIE Tokyo	
Kei Yuasa	OIE Reference Laboratory of KHVD National Research Institute of Aquaculture	
Yasuhiko Kawato	National Research Institute of Aquaculture	
Hyun Kwon	National Fishery Products Quality Management Service	
MARK CRANE	CSIRO – AAHL GEELONG AUSTRALIA	
Isabelle APZUL	IFREMER FRANCE	
Satu Viljamaa-Dirks	Finnish Food Safety Authority Evira	
Torunn Tolsedal	Norwegian Veterinary Institute	
KNUT FALK	NORWEGIAN VETERINARY INSTITUTE OSLO, NORWAY	
Ryan CARNEGIE	VIRGINIA INST. OF MARINE SCIENCE, USA	
NICK MOODY	CSIRO, AUSTRALIAN ANIMAL HEALTH LABORATORY	
Ikuo Igarashi	National Research Centre for Protozoan Diseases, Obihiro Univ. of Agr. and Vet Med., Obihiro JAPAN	
Hyoung Jun Kim	National Fishery Products Quality Management Service	

Background

Application of the OIE Twinning project between Korea and Denmark

National Veterinary Institute
Technical University of Denmark (DTU)
&
National Fishery Products Quality Management Service (NFQS)
of Ministry of Oceans and Fisheries (MOF)

16 February 2015

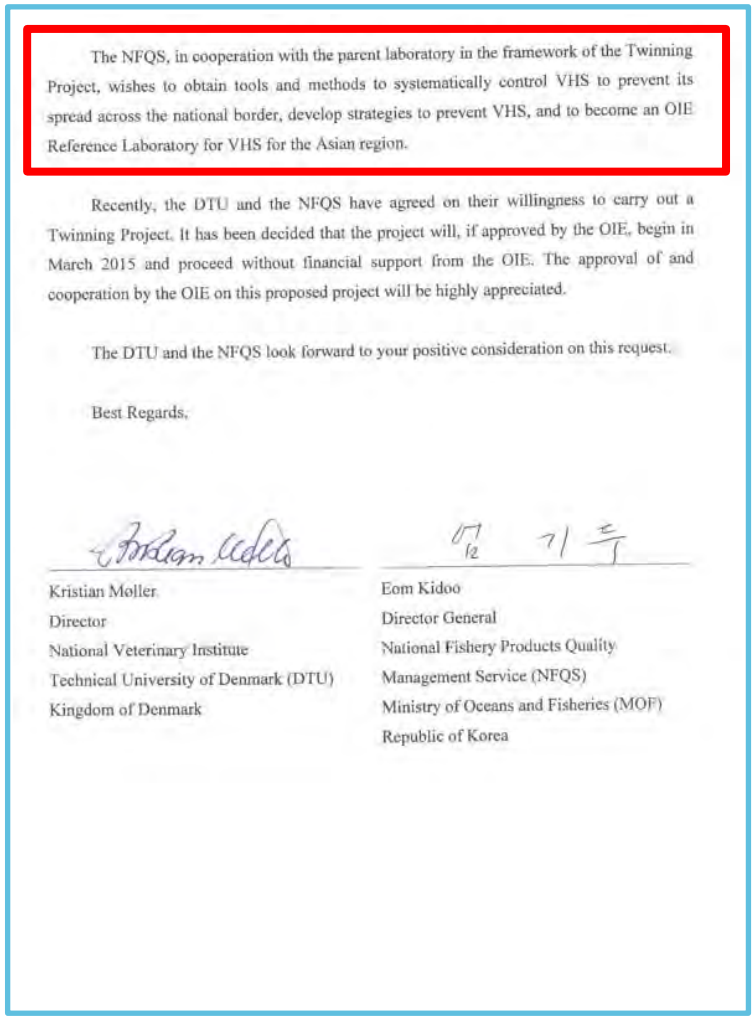
Dr. Keith Hamilton
Scientific and Technical Department
OIE Headquarters

Subject : Official letter signed by directors of both countries

Dear Dr. Keith Hamilton,

The National Veterinary Institute is a part of the Technical University of Denmark (DTU). The Fish Diseases Unit now placed in Copenhagen at the Section for Virology of the Institute is the National Reference Laboratory (NRL) for fish diseases in Denmark. Based on its research and control of VHS in Denmark, the institute was appointed as the European Union Reference Laboratory for Fish Diseases in 1994 and as the OIE Reference Laboratory for VHS the same year. After 50 years of control, the disease was eradicated from Denmark in 2009 and Denmark was approved as a VHS free EU Member State in 2013.

The National Fishery Products Quality Management Service (NFQS in South Korea, National Reference Laboratory for fish diseases) is the competent authority for the quarantine of internationally traded aquatic organisms, thereby contributing to improved quality of fishery products originated from Korea. While a significant proportion of olive flounders imported by the US, Japan and the EU is produced in aquaculture farms in Korea, outbreaks of VHS occur every year in Korea, and cause considerable damage for the aquaculture industry.



The NFQS, in cooperation with the parent laboratory in the framework of the Twinning Project, wishes to obtain tools and methods to systematically control VHS to prevent its spread across the national border, develop strategies to prevent VHS, and to become an OIE Reference Laboratory for VHS for the Asian region.

Recently, the DTU and the NFQS have agreed on their willingness to carry out a Twinning Project. It has been decided that the project will, if approved by the OIE, begin in March 2015 and proceed without financial support from the OIE. The approval of and cooperation by the OIE on this proposed project will be highly appreciated.

The DTU and the NFQS look forward to your positive consideration on this request.

Best Regards,

Kristian Møller
Director
National Veterinary Institute
Technical University of Denmark (DTU)
Kingdom of Denmark

Eom Kidoo
Director General
National Fishery Products Quality Management Service (NFQS)
Ministry of Oceans and Fisheries (MOF)
Republic of Korea

Background

Application of the OIE Twinning project between Korea and Denmark

Leader of OIE ref. lab in Denmark

CURRICULUM VITAE

Niels Jørgen Olesen

030655-0799, Sankt Annægade 34 2th., 1416 København K, Denmark.
Borne 3.6.1955 in Antwerp (Belgium). Moved to Denmark (Copenhagen) in 1964
Married in June 1984 to Iben Skov †24.09.2003
Three sons (borne 19.7.87, 29.6.1989, and 4.10.1991, respectively).

EDUCATION
Bachelor degree in mathematics and natural science, Øregaard Gymnasium, Copenhagen, 1974.
Awarded the Veterinarian degree from the Danish Royal Veterinary and Agricultural University, Copenhagen. Cand.med. Vet. 1982.
Danish PhD degree (Licentiatus Medicinae Veterinaria, lic.med.vet.) in Veterinary Science comprising tests in the subjects: Veterinary Virology (major subject), Immunology (auxiliary subject) and Epidemiology (auxiliary subject) and submitted the thesis: Egtved virus (viral haemorrhagic septicaemia virus): Specific antibodies in rainbow trout and in rabbits. The Royal Veterinary and Agricultural University. Lic.med vet. August 1987.

EMPLOYMENTS
Employed by the Co-operation Committee of the Danish Trout Industry at a research project concerning: Viral Haemorrhagic Septicaemia 1) Development of more sensitive methods in viral and serologic diagnosis 2) Attempts to intensify the eradication program of VHS in Denmark, 1982-1984.
Research Officer at the Danish Veterinary Laboratory, Department of Fish Diseases, Århus, Denmark, 1984-1989.
Senior Research Officer at the Danish Veterinary Laboratory, Department of Fish Diseases, Århus, Denmark, 1990-2011
Head of the Europen Union Reference Laboratory for Fish Diseases. www.eurl-fish.eu 1995-
Head of the OIE Reference Laboratory for Viral Haemorrhagic Septicaemia 1996-
Head of Section for Fish Diseases 2004-2006
Group leader 2006 –
DTU Professor in "Aquatic Animal Diseases", Technical University of Denmark, National Veterinary Institute (DTU Vet) June 2011–

STUDY TOURS
Institut Nationale de la Recherche Agronomique, Grignon, Paris, Frankrig.1 week, 1982.
Fish Disease Laboratory, Weymouth, England, 2 weeks, 1988.
Australia Animal Health Laboratory, Geelong, Australis, 1 week, 1997
Tamaki Station, Aquatic Animal Health division, National Research Institute of Aquaculture, Fisheries Research Agency, Tamaki, Mie, Japan. 1½ week Nov. 2009

Leader of OIE ref. lab accreditation project in Korea

Curriculum Vitae

♣ **Personal Data**

Name	Hyoung Jun KIM
Nationality	Republic of Korea
Sex	Male
Date of birth	May 16, 1978
Affiliation	National Fishery Products Quality Management Service
Address	106, haneulmaeulro, Ilsandong-gu, Goyang-si, Gyeonggi-do, 410-315, Korea Tel: 82-31-929-4784 CP: 82-10-6403-1882 E-mail:hjkim1882@korea.kr

♣ **Education**

1994.3-1997.2	Dong High School, Gunsan, Korea
1997.3-1999.7	Department of Marine Biomedical Science Kunsan National University, Korea
2002.3-2004.2	BS, Department of Aquatic Life Medicine Pukyong National University, Korea
2004.3-2006.2	MS, Department of Fish Pathology Pukyong National University, Korea
2006.4-2009.3	Ph.D, Graduate School of Fisheries Science and Technology Hokkaido University, Japan
2009.4-2009.6	Researcher Fisheries Science Institute Kunsan National University

Background

Application of the OIE Twinning project between Korea and Denmark

OIE Laboratory (or Collaborating Centre) Twinning Project Plan

1. Project plan[1], including:
   - 1.1. Overall project plan description
   - 1.2. Background
   - 1.3. A short and concise summary of the objectives
   - 1.4. Description of how the objectives will be met
   - 1.5. Reporting schedule (in accordance with the OIE Twinning Manual)
   - 1.6. A work plan showing who is involved in which task, including administration and budget management
   - 1.7. A training plan (if appropriate)
   - 1.8. Time Tables and measurable outputs (targets) for each stage
   - 1.9. Any foreseeable risks to the project
   - 1.10. A communication plan – including laboratory to laboratory or centre to centre, laboratory or centre to OIE, frequency of project updates/end stage reviews
   - 1.11. Where relevant, provisions for shipment of samples in accordance with the requirements for postage and packaging of biological materials described in the OIE *Terrestrial Animal Health Code* and in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*
   - 1.12. Overall budget estimate
   - 1.13. Complementary activities
2. Official letter(s) of mutual agreement signed by OIE Delegates of both OIE Member Countries concerned.
   NB: Can be provided at the end of the procedure before first transfer of funds
3. OIE Twinning Laboratories Manual – the Twinning partners are committed to abide by the provisions of this guide.

Parent Reference Centre	Candidate Centre
Signature of Parent Institute Representative	Signature of Candidate Institute Representative
Date: (dd/MM/yyyy) 17.02.2015	Date: (dd/MM/yyyy) 16/02/2015

Background

Application of the OIE Twinning project between Korea and Denmark

농 림 축 산 식 품 부
Ministry of Agriculture, Food and Rural Affairs
94, Dasom 2-RO, Sejong-Si, Republic of Korea, 339-012
Tel: +82-44-201-2351, FAX: +82-44-868-0628
E-mail: animalhealth@korea.kr; ceo0210@korea.kr

Dr. Keith Hamilton
Scientific and Technical Department
OIE Headquarters

12 February 2015

RECOMMENDATION LETTER FOR OIE LABORATORY TWINNING PROJECT APPLICATION

Dear Dr. Keith Hamilton,

I reviewed the application for an OIE Laboratory Twinning project for viral haemorrhagic septicaemia (VHS) disease between the National Veterinary Institute (DTU, Denmark, the OIE Reference Laboratory for VHS) and the National Fishery Products Quality Management Service (NFQS, South Korea, the National Reference Laboratory for fish diseases.)

The NFQS is the competent authority for quarantine of internationally traded aquatic organisms, thereby contributing to improved quality of fishery products originated from Korea. While meaningful proportion of olive flounders imported by the US, Japan and the EU is produced in aquaculture farms in Korea, outbreaks of VHS in Korea, which occur every year in Korea, cause considerable damage for the aquaculture industry. In this context, the NFQS, in cooperation with the parent laboratory in the framework of the Twinning project, wishes to obtain tools and methods to systematically control VHS to prevent its spread across the national border, develop ways to prevent VHS, and become an OIE Reference Laboratory for VHS for the Asian region.

I believe the overall objective of this project is a good fit with the objective of the Twining Programme, which is to provide a more even geographical distribution of OIE Reference Laboratories.

I therefore fully support this Twinning project, and wish that the OIE considers positively approving this project.

Sincerely yours,

Oh Soon-min

Chief Veterinary Officer, D.V.M.
Director of General Animal Health Division
Ministry of Agriculture, Food and Rural Affairs
94, Dasom 2-RO, Sejong-City, 339-012
Republic of KOREA

Ministry of Food, Agriculture and Fisheries of Denmark
The Danish Veterinary and Food Administration

World Organisation for Animal Health
Scientific and Technical Department
12 rue de Prony
75017 Paris
France

Att. Keith Hamailton

Date: 17. April 2015

Dear Sirs,

I hereby confirm that I as the Danish CVO and OIE delegate support the twinning project initiated between the National Reference Laboratory (NRL) for fish diseases in Korea at the National Fishery Products Quality Management Service (NFQS) of the Republic of Korea, as the candidate institute, and the National Veterinary Institute, DTU, Denmark as the parent institute being the European Union Reference Laboratory for Fish Diseases (EURL) and the OIE Reference Laboratory for VHS.

The candidate laboratory wants to improve the capabilities of performing its duties as the Korean NRL for Viral Haemorrhagic Septicaemia (VHS). At the end of the twinning project the NFSQ will apply for obtaining a status as OIE Reference Laboratory or OIE Collaborating Centre for fish diseases with focus on VHS for the Asian region.

Yours sincerely

Per Henriksen
Chief Veterinary Officer

Background

Application of the OIE Twinning project between Korea and Denmark

TR: RE: Proposal for an OIE Twinning project on VHS between Denmark and Korea

90.80.168.145 FR(프랑스) 신고하기

보낸사람: Gounalan Pavade <g.pavade@oie.int> 주소추가 수신거부

받는사람: "hjkim1882@korea.kr" <hjkim1882@korea.kr> 주소추가

참조: Keith Hamilton <k.hamilton@oie.int> 주소추가

보낸날짜: 2015년 03월 18일 01시 45분 42초

보안단계 1 | 2 | 3 | 4 | 5 해당 본문으로 메일쓰기

Dear Hyoung Jun Kim,

The twinning project was presented to the Aquatic Commission in the first week of March for technical comments. The Commission is in favour of this twinning project to be initiated between Denmark and Republic of Korea for Viral Haemorrhagic Septicaemia (VHS).

The Commission also commented that the objective of this twinning project should concentrate on increasing the diagnostic capacities of the candidate lab (i.e National Fishery Products Quality Management Service, Republic of Korea). The twinning project should not aim to become a research project to characterise the VHS virus isolates in the region.

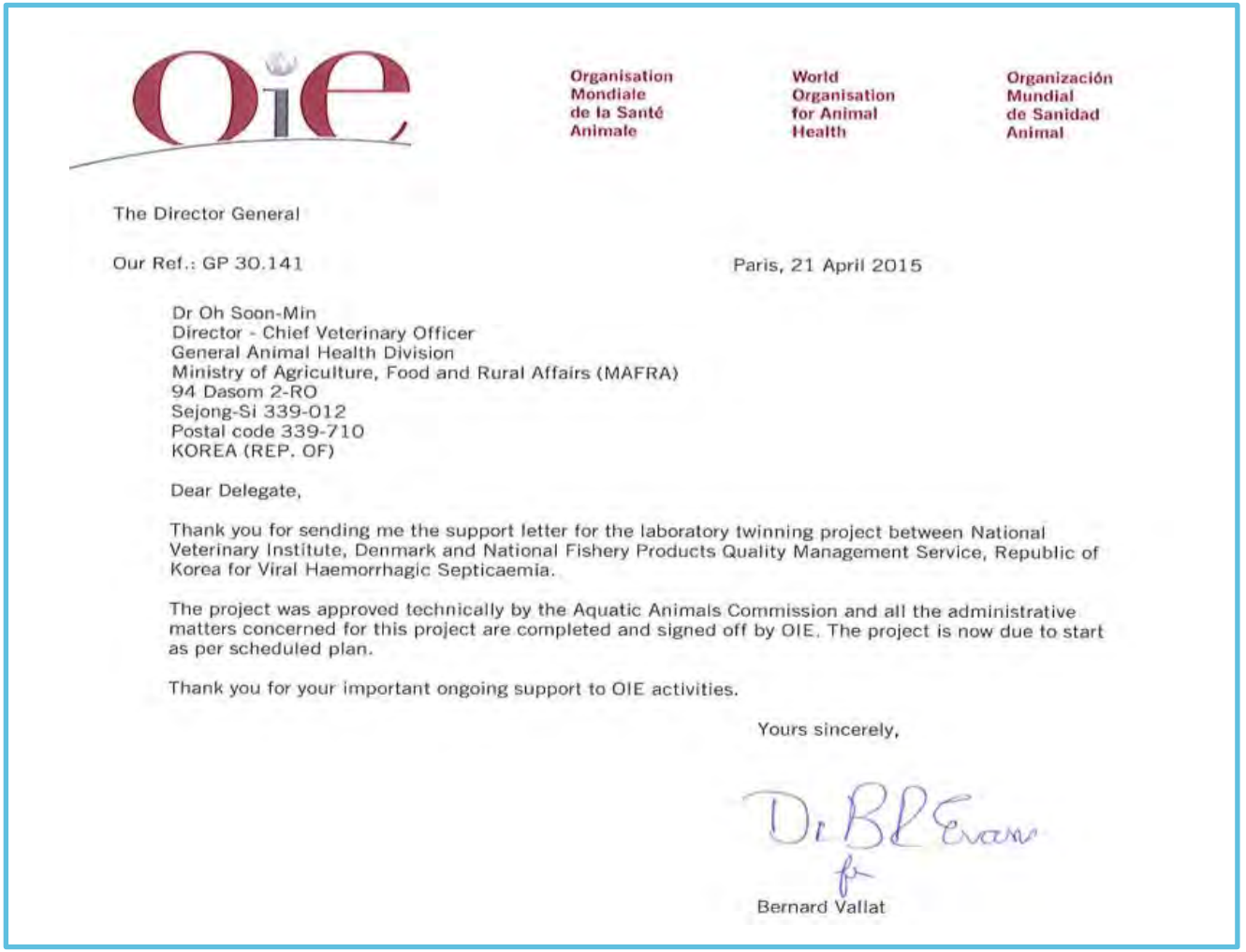
As a final step, before the project could start, we would need the support letters from the OIE Delegates of Denmark and South Korea on this project.

Then I will get the final approval on the twinning contract by our Director General.

Regards,
Gounalan.

Background

Approval from OIE headquarters



Organisation Mondiale de la Santé Animale
World Organisation for Animal Health
Organización Mundial de Sanidad Animal

The Director General

Our Ref.: GP 30.141

Paris, 21 April 2015

Dr Oh Soon-Min
Director - Chief Veterinary Officer
General Animal Health Division
Ministry of Agriculture, Food and Rural Affairs (MAFRA)
94 Dasom 2-RO
Sejong-Si 339-012
Postal code 339-710
KOREA (REP. OF)

Dear Delegate,

Thank you for sending me the support letter for the laboratory twinning project between National Veterinary Institute, Denmark and National Fishery Products Quality Management Service, Republic of Korea for Viral Haemorrhagic Septicaemia.

The project was approved technically by the Aquatic Animals Commission and all the administrative matters concerned for this project are completed and signed off by OIE. The project is now due to start as per scheduled plan.

Thank you for your important ongoing support to OIE activities.

Yours sincerely,

Dr B P Evans
p/
Bernard Vallat

Background

Report to Vice-minister of ministry of Oceans and Fisheries

사무관	과 장	원 장	수산정책실장	차 관
김용득	권현욱	임기두		김영석
협조 : 어촌양식정책관 오용연				

수산생물질병분야

OIE 표준실험실 인정 추진 계획

2015. 6.

FIQ 국립수산물품질관리원

Project Procedure to Date

2015

☞ 2015. 7. Kick-off meeting in Copenhagen


☞ 2015. 9. Education of diagnostic method to Ecuador inspector (ODA)

Project Procedure to Date

☞ 2015. 10.

- Obtaining the exclusive budget ($250,000/yr) for the OIE works
- Discussion and meeting on improvement of diagnostic tools for VHSV in EAFP international conference

Dr. Taksdal

Dr. Satu

Dr. Bergmann

Meeting for Twinning project

Project Procedure to Date

☞ 2015. 10-11. International proficiency test from EU reference laboratory for fish

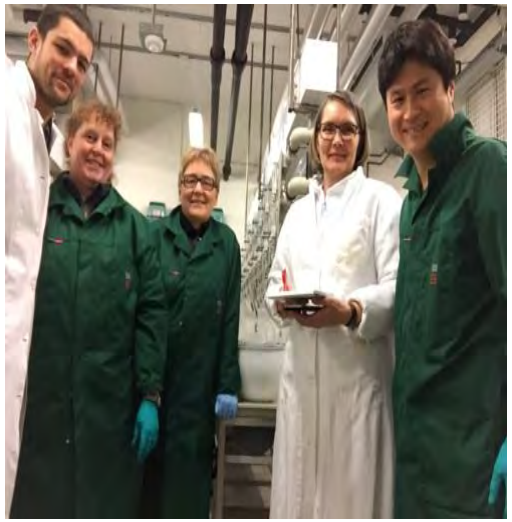
☞ 2015. 11. Meeting on the research for VHS diagnosis in Korea (TAIEX)

☞ 2015. 12. Submission for ISO 17025 laboratory accreditation of VHS

Project Procedure to Date

☞ 2015. 12 – 2016. 2.

- Cooperation research in Copenhagen for 7 weeks
  (for development of VHSV detection method using conventional PCR)
- meetings and final presentation for the research

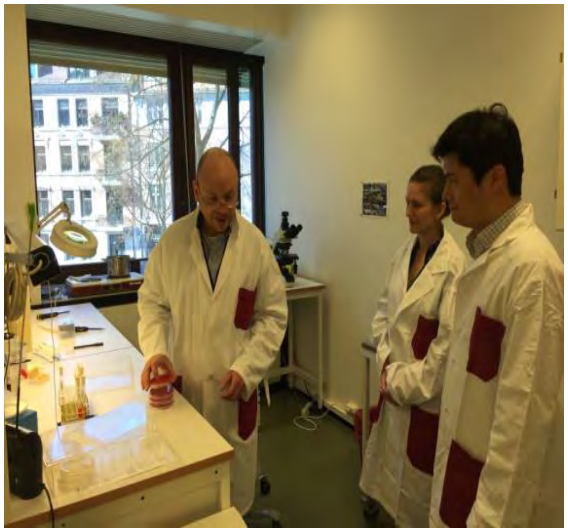


Project Procedure to Date

2016

☞ 2016. 3. Additional cooperation research in Copenhagen for 1 week

☞ 2016. 4. Training course for OIE ref. lab. in Norway



Project Procedure to Date

☞ 2016. 4. Meeting on the research for VHSV diagnosis in Korea
1st International workshop on VHS in Korea

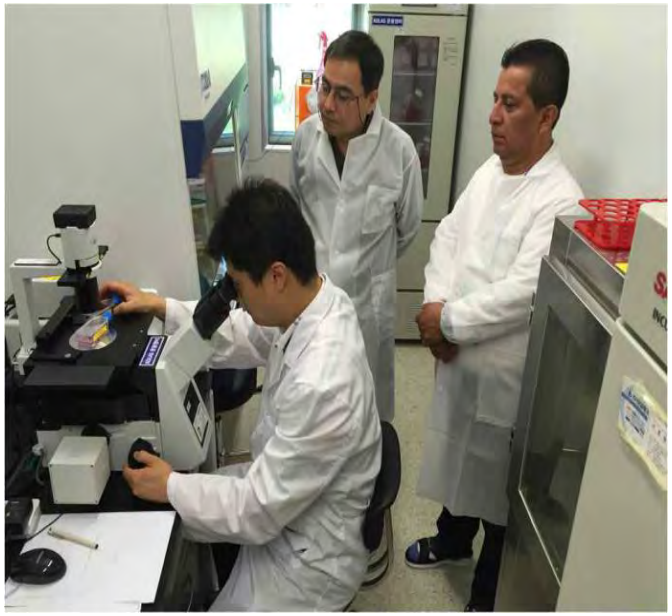
☞ 2016. 5. Meeting with president (Dr. Ingo Ernst) and member (Dr. Joanne Constantine) about OIE Twinning project between Korea and Denmark


Dr. Ingo Ernst


Dr. Joanne Constantine

Project Procedure to Date

☞ 2016. 8. Second education of diagnostic methods to Ecuador inspector (ODA)



Project Procedure to Date

☞ 2016. 8. Obtained the ISO 17025 on VHS diagnostic methods
(Cell culture, Molecular techniques)

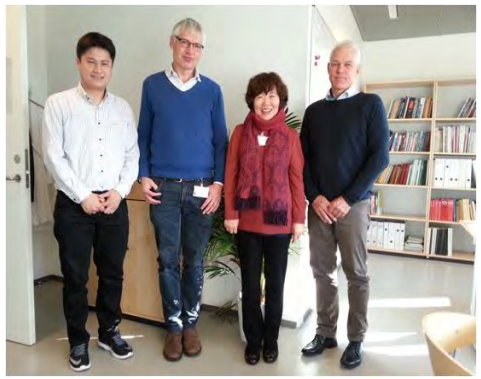
☞ 2016. 8. Submission of the annual report to the OIE headquarters

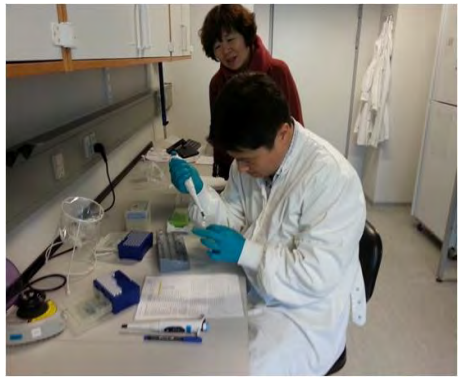
Project Procedure to Date

☞ 2016. 9. Transfer of all VHSV genotypes from the OIE reference laboratory (DTU) on VHS to NFQS

25 VHSV isolates of all genotypes

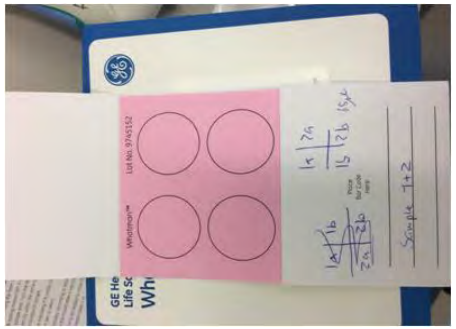
☞ 2016. 10. Meeting for OIE twinning project between Korea and Denmark in DTU
- The name of new conventional RT-PCR method for VHSV detection : 3F2R





Project Procedure to Date

☞ 2016. 10. Preparation of FTA cards for proficiency test using new 3F2R method

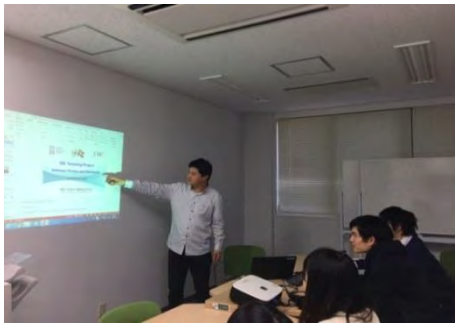


FTA cards for virus samples

Prepared FTA cards for delivery to several institutes

☞ 2016. 10-11. International proficiency test from DTU

☞ 2016. 12-2017. 1. Training to OIE regional representation for Asia and the Pacific



Project Procedure to Date

2017

☞ 2017. 1. Proficiency test for check the reproducibility using 3F2R method
- CEFAS(UK), ANSES(France), IZSVe(Italy), FLI(Germany), FRA(Japan) DTU(Denmark), NFQS(Korea)

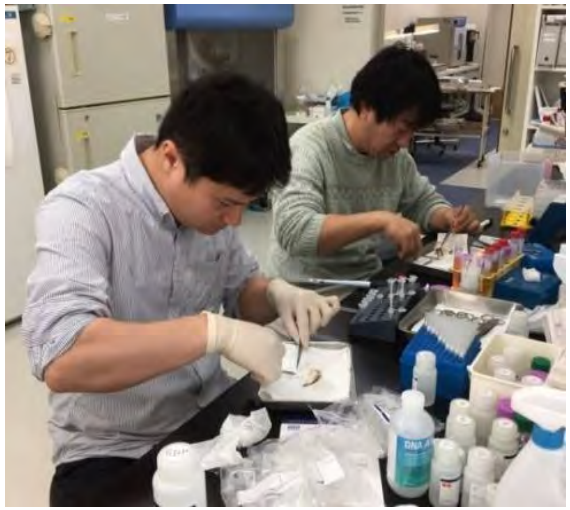
☞ 2017. 2. Consultations with OIE Tokyo office for activation of OIE activities and discussion about 3F2R method with Dr. Crane and Dr. Moody (OIE experts)

Group meeting of OIE aquatic animal experts

Discussion about novel VHSV detection method (KIM3F2R)

Project Procedure to Date

☞ 2017. 2. Cooperation research with OIE reference laboratory for KHV (Japan)
- checked the reproducibility for VHSV detection using 3F2R
- standardization for fish diagnostic method using gene detection



Co-research for KHV

Discussion with members of
fish diagnostic center in Japan

Project Procedure to Date

☞ 2017. 3. Meeting with general directors between NFQS and NVI (DTU)

☞ 2017. 3. Experimental discussion with Ministry for primary industries of New Zealand at the laboratory of NFQS

Project Procedure to Date

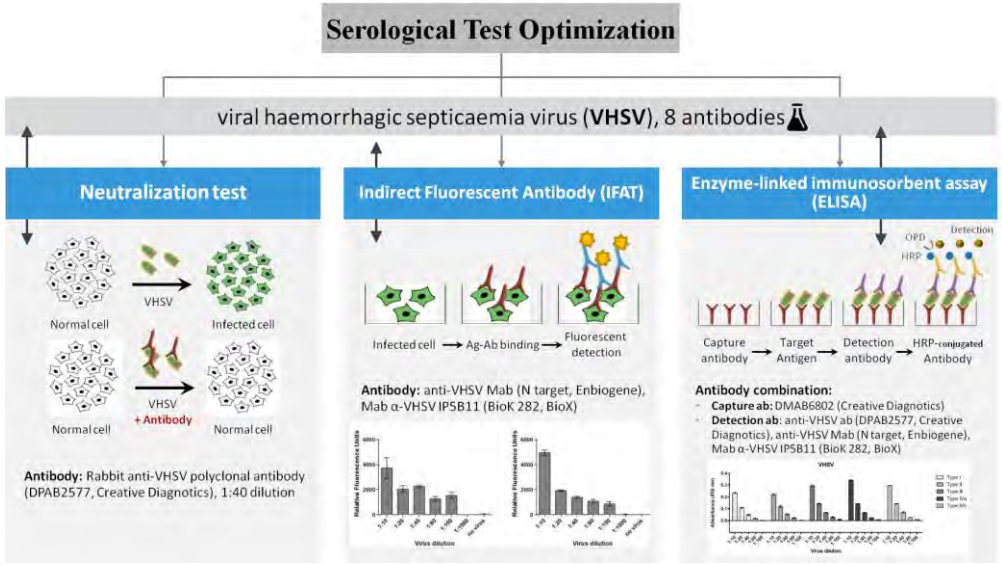
☞ 2017. 3. Course on OIE and OIE Twinning Project to representatives from 16 countries recipients of ODA by KOICA (March 2017)

Presentation about the OIE Twinning Project to students and researchers from 16 ODA recipient countries

Participants of the course in front of the NFQS headquarters

Project Procedure to Date

☞ 2017. 4. Optimized the serological methods for VHSV detection
- neutralization method, ELISA and IFAT for VHSV detection in NFQS
  ♣ finished to prepare all VHSV diagnostic methods in OIE manual



The confirmatory diagnostic methods for VHS were all prepared in NFQS.
(Cell culture, Antibody-based assays, conventional RT-PCR followed by sequencing and Real-time RT-PCR method)

Project Procedure to Date

☞ 2017. 5. 2nd International Workshop for VHS and rhabdoviral disease in Korea
- 40 people (Dr. Olesen from Denmark, Dr. Garver from Canada, Dr. Panzarin from Italy, 7 Korean experts including FMD OIE reference laboratory expert, other government researchers and graduate students)
- 16 topics and comprehensive discussion

Project Procedure to Date

☞ 2017. 5. Scientific meeting of VHS experts and tour of the aquatic animal quarantine (AAQ) laboratory in NFQS

AAQ laboratory tour with Dr. Olesen and Dr. Garver

Discussion about the OIE diagnostic manual and the results of the new diagnostic tool for VHS

Project Procedure to Date

☞ 2017. 5. Participation in OIE General Assembly and Aquatic Commission pre-meeting

Group photo of the Korean delegation

Aquatic Commission pre-meeting

Project Procedure to Date

☞ 2017. 5. Participation at OIE general assembly and discussion with Dr. Ingo and members of aquatic animal commission about OIE Twinning Project between Korea and Denmark

☞ 2017. 5. Teams meeting of OIE Twinning projects between IHN (China & USA) and VHS (Korea & Denmark) at the OIE general assembly

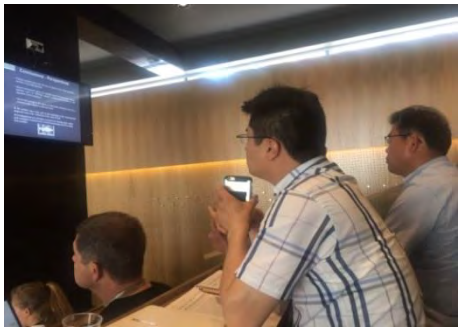
Information exchange between Dr. Hong Liu and Dr. Kim

Photo with Dr. Hong Liu in OIE general assembly

Project Procedure to Date

☞ 2017. 5. Oral presentation (Novel 3F2R method) on EURL annual workshop at Copenhagen

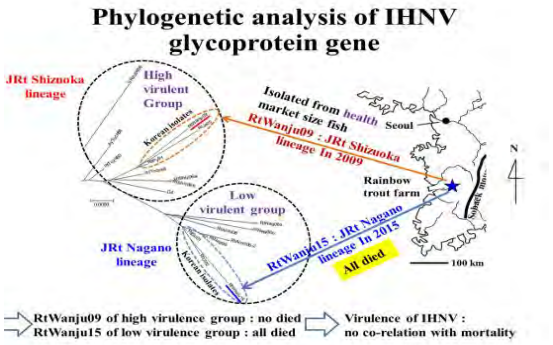

☞ 2017. 6. Oral presentation (Novel 3F2R method) on ISVLV international conference in Budapest

Project Procedure to Date

☞ 2017. 6. Discussion of Dr. Kim and Dr. Kurath on the Korean IHNV at the ISVLV

Photo of Dr. Gael Kurath and Dr. Kim



Discussion about research data of Korean IHNV

Project Procedure to Date

☞ 2017. 7. Submission of annual report of OIE Twinning project to OIE headquarters

☞ 2017. 8. Oral presentation at OIE Twinning Project workshop between Japan and Indonesia on koi herpesvirus in Bali

- Presentation about the status of the OIE Twinning project between Korea and Denmark
- The status of KHV research in Korea

Project Procedure to Date

☞ 2017. 9. Oral presentations (3 Topics) at the 18th International Conference on disease of fish and shellfish in Belfast

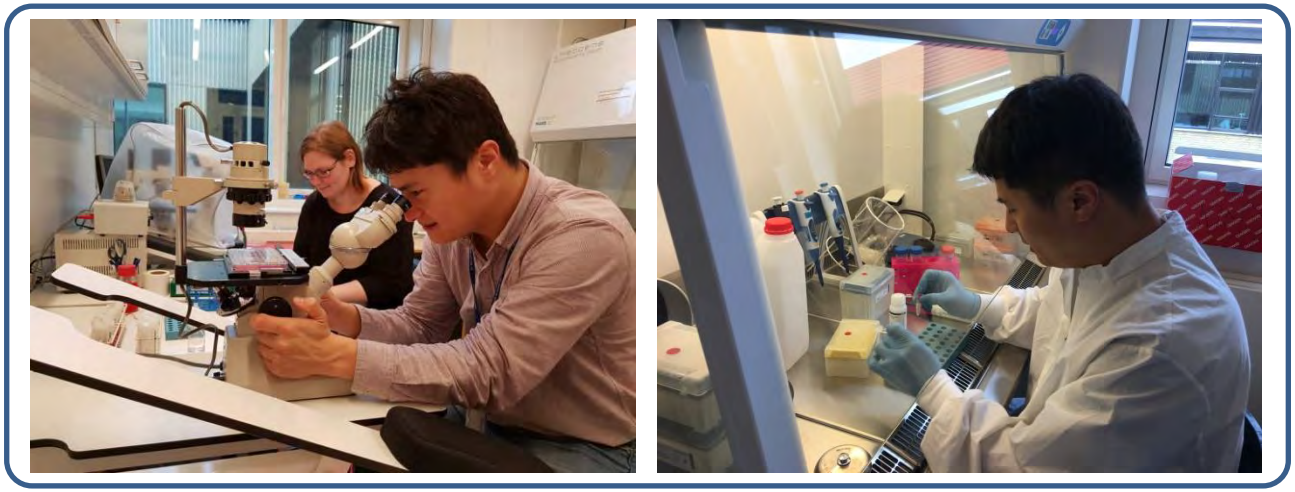
- Detection of viral DNA, mRNA and infectivity in koi fin (KF-1) cells infected with different concentration of KHV
- Comparison of susceptibility of KHV between koi carp and ginbuna
- Development and validation of a novel RT-PCR method for VHSV detection

Project Procedure to Date

☞ 2017. 10. Meeting for summary about activities of OIE Twinning Project in Denmark

Project Procedure to Date

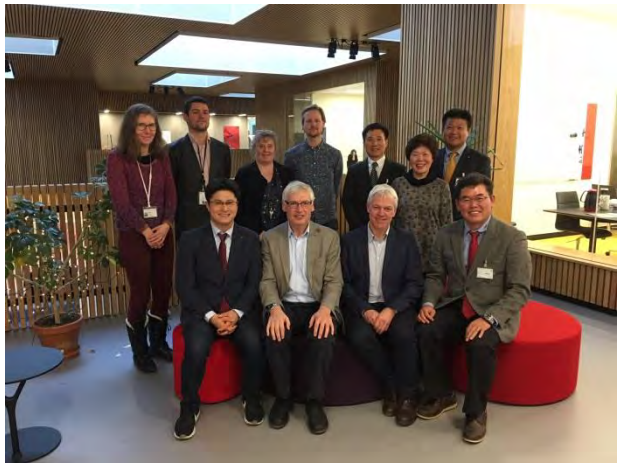
☞ 2017. 9 ~ 11. Cooperation research about Korean IHNV and new diagnostic method for VHS in Denmark for 7 weeks



Project Procedure to Date

☞ 2017. 12. Finalization of OIE Twinning Project between Korea and Denmark


☞ 2017. 12. Memorandum of Agreement (MOA) between NFQS and NVI of Denmark

☞ 2017. 12. Submission of Proposal for amendments in the Chapter 2.3.10 on VHS in the OIE Aquatic Manual

Aquatic Animals Health Standards Commission

OIE Organisation Mondiale de la Santé Animale

World Organisation for Animal Health
12, rue de Prony 75017 Paris, France

Kgs Lyngby December 18th 2017

To the OIE Aquatic Commission,

Proposal for amendments in the Chapter 2.3.10 on Viral haemorrhagic septicaemia (VHS) in the OIE Aquatic Manual

There is a serious need to make some changes in Chapter 2.3.10 Viral Haemorrhagic Septicaemia in the Manual of Diagnostic Tests for Aquatic Animals, 7th Edition. The changes are important due to the fact that the conventional RT-PCR for detection of VHSV given in the chapter do not react or react poorly with VHSV genotype IVa, one of the major genotypes of this virus, in addition the RT-PCR presently recommended in the Manual often gives false positive reactions with fish cell cultures. Therefore significant efforts have been put into the development and validation of a conventional RT-PCR that react equally well with all genotypes of VHSV and that do not produce false positive amplicons. This work was conducted as part of an OIE Twining project between the OIE Reference Laboratory for VHS at DTU in Denmark and the National Fishery Products Quality Management Service in South Korea. The study was submitted in October 2017 for publication in Aquaculture and is expected to be published first 2018 (manuscript attached as Annex 2).

The validation was performed according to the given OIE recommendation and the new method was shown to be as sensitive and specific as a previously validated real-time RT-PCR (Jonstrup et al. 2013) and as the commonly used cell culture methods for VHSV.

We therefore recommend that the method described in the present chapter is replaced by this new method, as proposed in the table below where the changes are highlighted.

In addition we would like to use this occasion to recommend that surveillance for VHS can be conducted by traditional cell culture technique or by real-time RT-PCR as described in Jonstrup et al 2013. This real-time RT-PCR was fully validated and tested according to OIE protocols and was subsequently examined in a large survey conducted in US (Janet Warg et al 2014a and Warg et al. 2014b) concluding that the Jonstrup et al. one step protocol is the most sensitive, specific and robust PCR based method and should be recommended for surveillance of VHSV. The other real-time RT-PCR described by Garver et al. and presently given in the Manual is almost as sensitive and specific as the Jonstrup et al. RT-PCR but is a 2-step method that should not be recommended for surveillance purpose due to risks of cross contaminations.

Finally we would like to remove the recommendations of using the direct immunochemical methods (ELISA and IFAT) on fish tissue for surveillance purpose as these methods cannot meet the expected requirements for sensitivity and specificity.

The key references to these proposals for amendments are attached and we sincerely hope that the changes can be done within short time as the need for changing especially the conventional RT-PCR given in the VHS chapter of the OIE Aquatic Manual is acute.

Yours sincerely

Hyoung Jun Kim and Niels Jørgen Olesen

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National Fishery Products Quality Management Service,
337, Haeyang-ro, Yeongdo-gu, Busan, 606-080, Republic of Korea

Activities (2015-2017) of OIE Twinning Project between Korea and Denmark

Research

Research Paper
- International : 6
- Domestic : 2

Book for aquatic diseases : 1

Patent : 3

Poster Presentation : 9
- International : 7
- Domestic : 2

Oral Presentation (2016~2017)
- International : 16

Setting of laboratory

ISO/IEC 17025
- Molecular techniques
- Cell culture method

Serological tests
- Neutralization test
- ELISA
- IFAT

Laboratory Setting by OIE standard

International activities

Scientific meeting : 25 times

Collaboration agreements : 5

Proficiency tests (EU) : 4 times

OIE conference : 8 times
- General Assembly : 5
- Focal point seminar : 1
- Reference Lab : 2

International Workshop : 4
- EURL workshop : 1
- NFQS workshop : 2
- Japan-Indonesia : 1

Project Procedure to Date

Application for designation

as an OIE Reference Laboratory
for Viral Haemorrhagic Septicaemia

December 2017

National Fishery Products Quality Management Service
Ministry of Oceans and Fisheries

Republic of Korea

Table of Contents

Organisation Mondiale de la Santé | World Organisation for Animal | Organización Mundial de Sanidad

http://www.oie.int/scientific-expertise/reference-laboratories/list-of-laboratories/

List of Laboratories: OIE - W...

Viral haemorrhagic septicaemia

Dr Kyle Garver
Pacific Biological Station – Aquatic Animal Health Laboratory (PBS-AAHL)
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3190 Hammond Bay Road
Nanaimo V9T 6N7
British Columbia
CANADA
Tel: +1-250 756 73 40 Fax: +1-250 756 70 53
Email: Kyle.Garver@dfo-mpo.gc.ca

Dr Niels Jørgen Olesen
National Veterinary Institute
Technical University of Denmark
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Tel: +45 35 88 68 31 Fax: +45 35 88 69 01
Email: njol@vet.dtu.dk

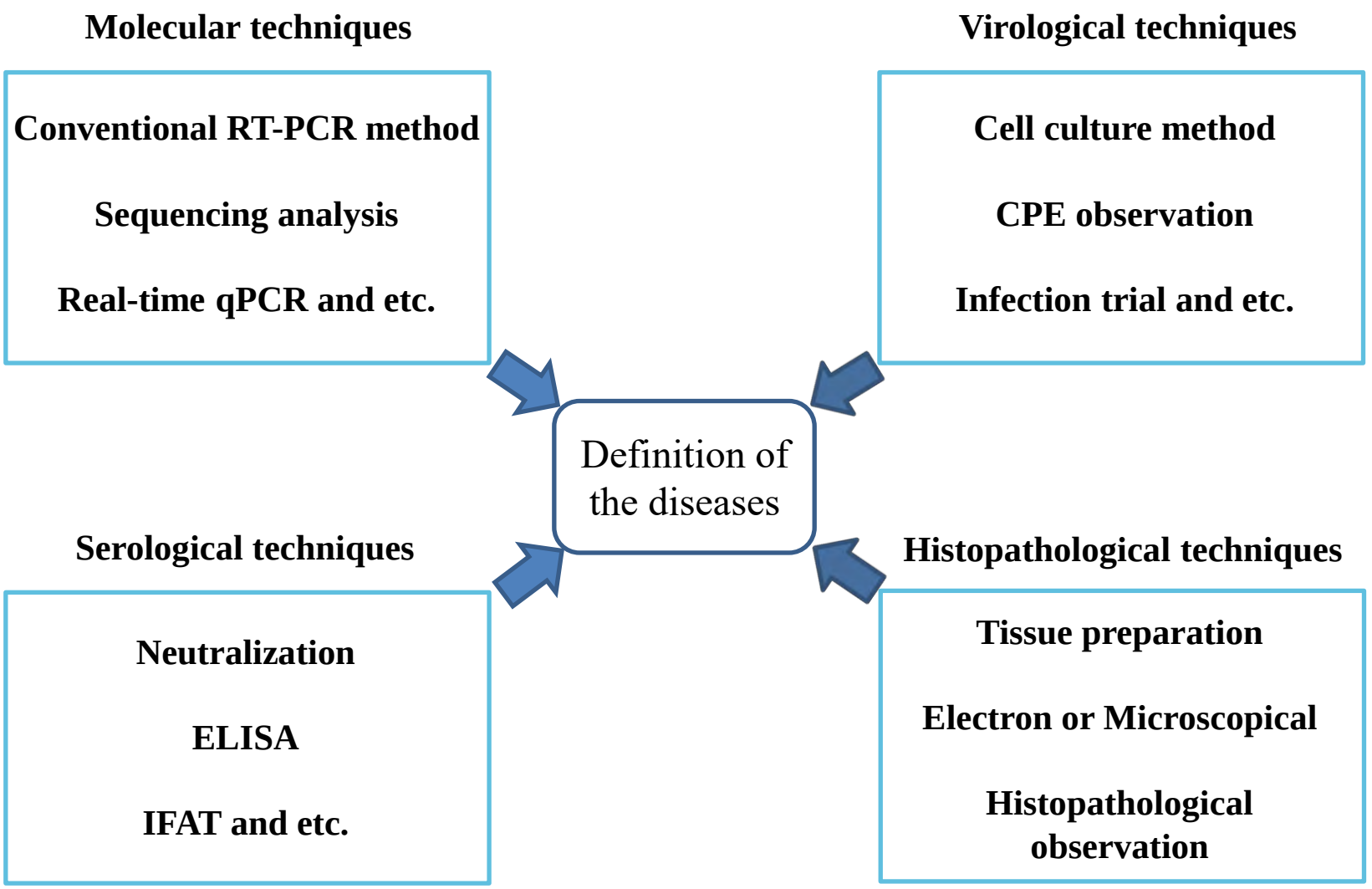
Dr Hyoung Jun Kim
Aquatic Animal Quarantine (AAQ) Laboratory
General Service Division
National Fishery Products Quality Management Service (NFQS)
Ministry of Oceans and Fisheries
337 Haeyang-ro
Yeongdo-gu
Busan, 49111
KOREA (REP. OF)
Tel: +82-51 400 56 53 Fax: +82-51 400 56 55
Email: hjkim1882@korea.kr

cc: Dr Hyoung Jun Kim, Dr Matthew Stone, Dr Elisabeth Erlacher-Vindel

Terms of OIE reference laboratory

- To use, promote and disseminate diagnostic methods validated according OIE Standards (confirmatory diagnosis)
- To develop reference material in accordance with OIE requirements, and implement and promote the application of OIE Standards
- To develop, standardise and validate according to OIE Standards new procedures for diagnosis and control of the designated pathogens or diseases;
- To provide diagnostic testing facilities, and, where appropriate, scientific and technical advice on disease control measures to OIE Member Countries
- To carry out and/or coordinate scientific and technical studies in collaboration with other laboratories, centres or organisations
- To collect, process, analyse, publish and disseminate epizootiological data relevant to the designated pathogens or diseases
- To provide scientific and technical training for personnel from OIE Member Countries
- To organise and participate in scientific meetings on behalf of the OIE
- To maintain a system of quality assurance, biosafety and biosecurity relevant for the pathogen and the disease concerned
- To establish and maintain a network with other OIE Reference Laboratories designated for the same pathogen or disease and organise regular inter-laboratory proficiency testing to ensure comparability of results
- To organise inter-laboratory proficiency testing

Standards of OIE diagnostic manual for definite diagnosis of VHS



Detail plans of new OIE reference laboratory for VHS

Activities for employ of experts (Serologist, Histo-pathologist, Molecular biologist)

Open Homepage (website) for OIE reference laboratory

International Education Program (September every year)

Cooperation research with OIE reference laboratories

Research for validation of diagnostic tools for aquatic animal diseases

Establishment of facility for infection trial

Participation and host of Proficiency test

Host the International Workshop every year

Development of a novel one-step reverse transcription PCR method for detecting viral haemorrhagic septicaemia virus

H. J. Kim[1]*, A. Cuenca[2] & N. J. Olesen[2]

[1]National Fishery Products Quality Management Service, Busan, Korea
[2]National Veterinary Institute, Technical University of Denmark, Frederiksberg C, Denmark

OIE Twinning project on VHS between Denmark and Korea

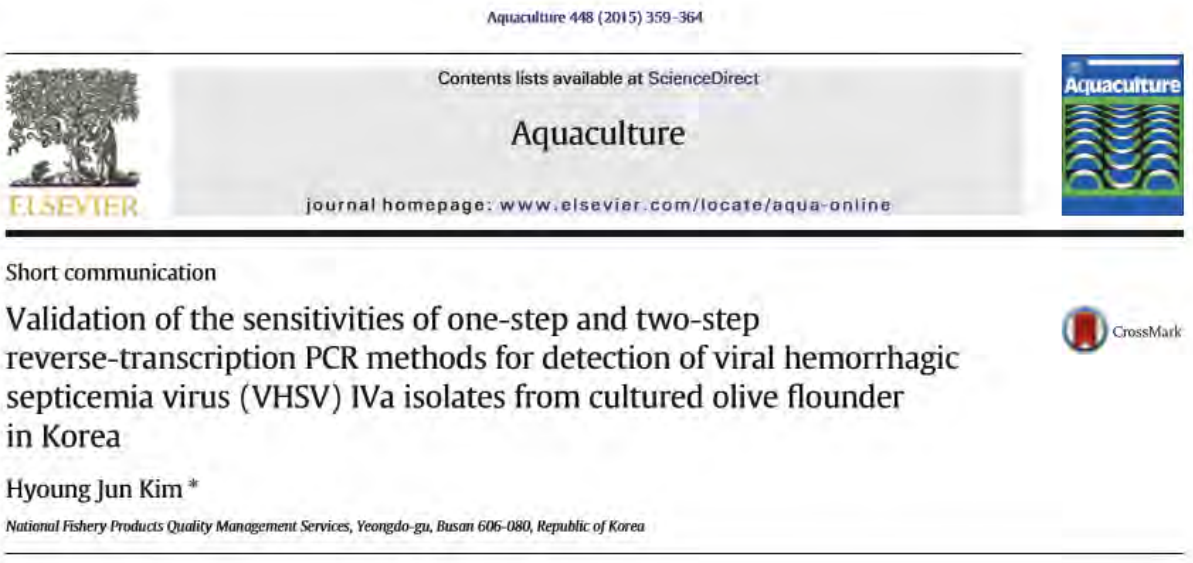
Background

- **Conventional PCR** is regularly used for **detection and genotyping of pathogens.**

Background

- **Conventional PCR** is regularly used for **detection and genotyping of pathogens.**

- However, I found a **low sensitivity (10,000 folds)** for detection of **VHSV IVa isolates** using the conventional RT-PCR described in the current OIE aquatic manual (VN primer set).



Aquaculture 448 (2015) 359–364

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

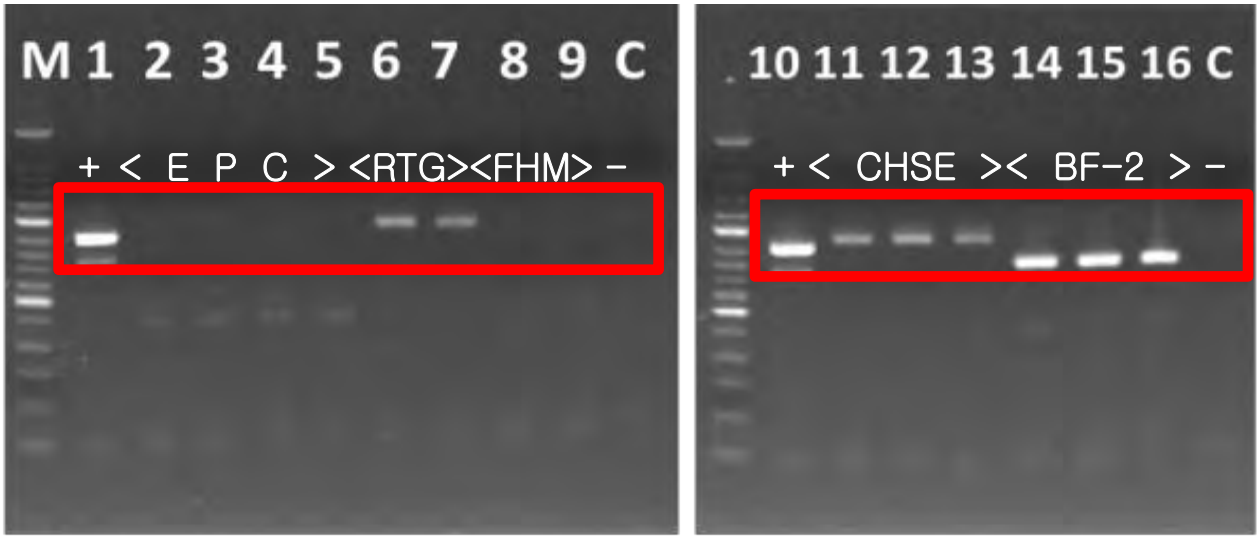
Short communication

Validation of the sensitivities of one-step and two-step reverse-transcription PCR methods for detection of viral hemorrhagic septicemia virus (VHSV) IVa isolates from cultured olive flounder in Korea

Hyoung Jun Kim *

National Fishery Products Quality Management Services, Yeongdo-gu, Busan 606-080, Republic of Korea

Background



- And, **non-specific bands with fish cell lines** were often observed when using the OIE RT-PCR.

  - In particular, these non-specific bands showed sizes **very close to the positive VHSV control bands**.

Background

- **Conventional PCR** is regularly used for **detection and genotyping of pathogens.**
- However, we found a **low sensitivity** for detection of **VHSV IVa isolates** using the conventional RT-PCR described in the OIE aquatic manual (VN primer set).
- And, **non-specific bands with fish cell lines** were often observed when using the OIE RT-PCR.
- Thus, a **novel conventional RT-PCR (3F2R)** have been developed and validated for detection of all genotypes of VHSV.

For New Primer Design

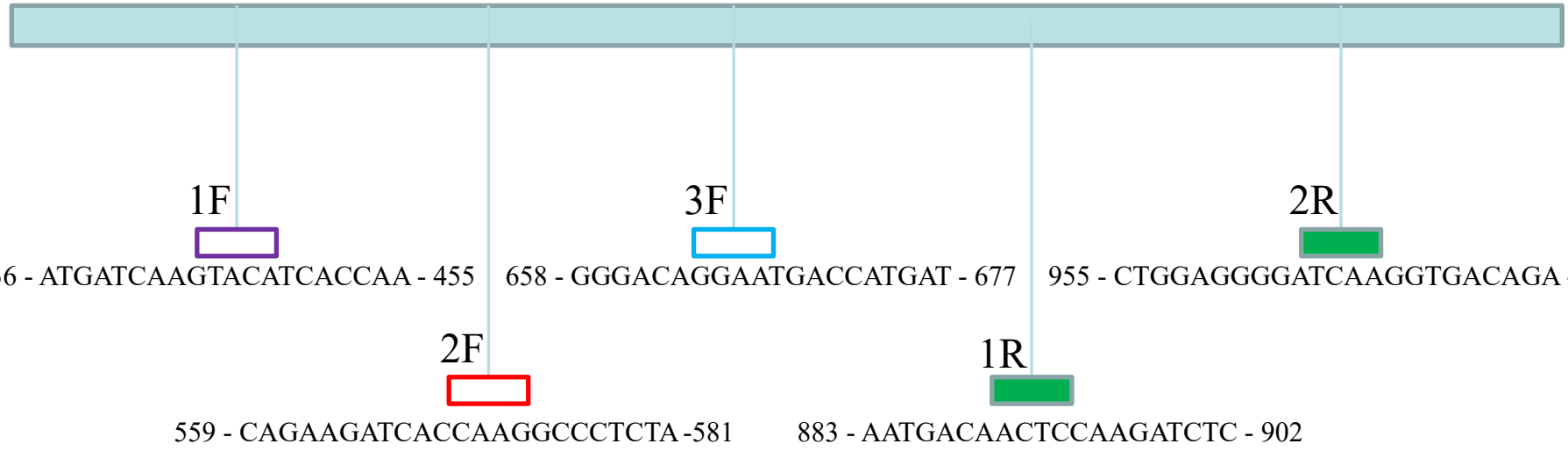
- Investigation of primer sets for VHSV gene detection in **37 published articles**.
  - Result : **No primer set matched** all VHSV genotypes

- **Candidate primers** for 5 regions were designed using 136 VHSV **N gene** from NCBI and EURL **Genbanks**.

1

VHSV N gene ORF

1215



1F
436 - ATGATCAAGTACATCACCAA - 455
436 - ATGATCAAGTACATCA**A**CAA - 455 BC06-89-1 isolate (only)
436 - ATGATCAAGTACATCAC**T**AA - 455 Fi13 isolate (only)

3F
658 - GGGACAGGAATGACCATGAT - 677
658 - GGGAC**G**GGAATGACCATGAT - 677
two isolates : 99-292, BC02-235

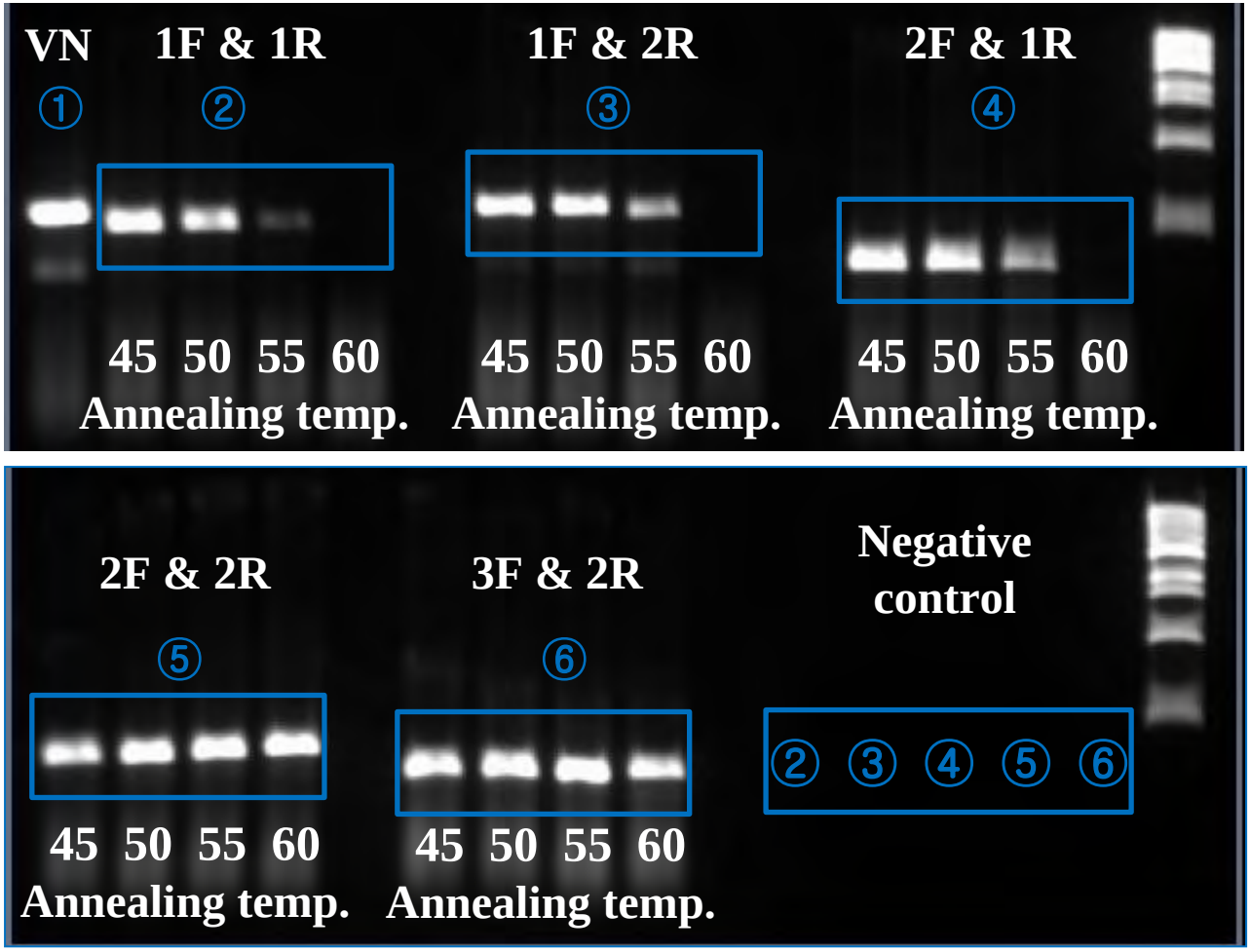
2F
559 - CAGAAGATCACCAAGGCCCTCTA -581
559 - CAGAAGATCAC**A**AAGGCCCTCTA -581 GH40 isolate (only)
559 - CAGAAGATCACCAA**A**GCCCTCTA -581 VHS IVa KJ2008 (only)

RT-PCR using 5 primer sets

1 set : 1F & 1R = 456 bp
2 set : 1F & 2R = 541 bp
3 set : 2F & 1R = 418 bp
4 set : 2F & 2R = 343 bp
5 set : 3F & 2R = 319 bp

: Complete match with all isolates

VHSV RT-PCR: 5 primer sets tested at 4 annealing temperatures

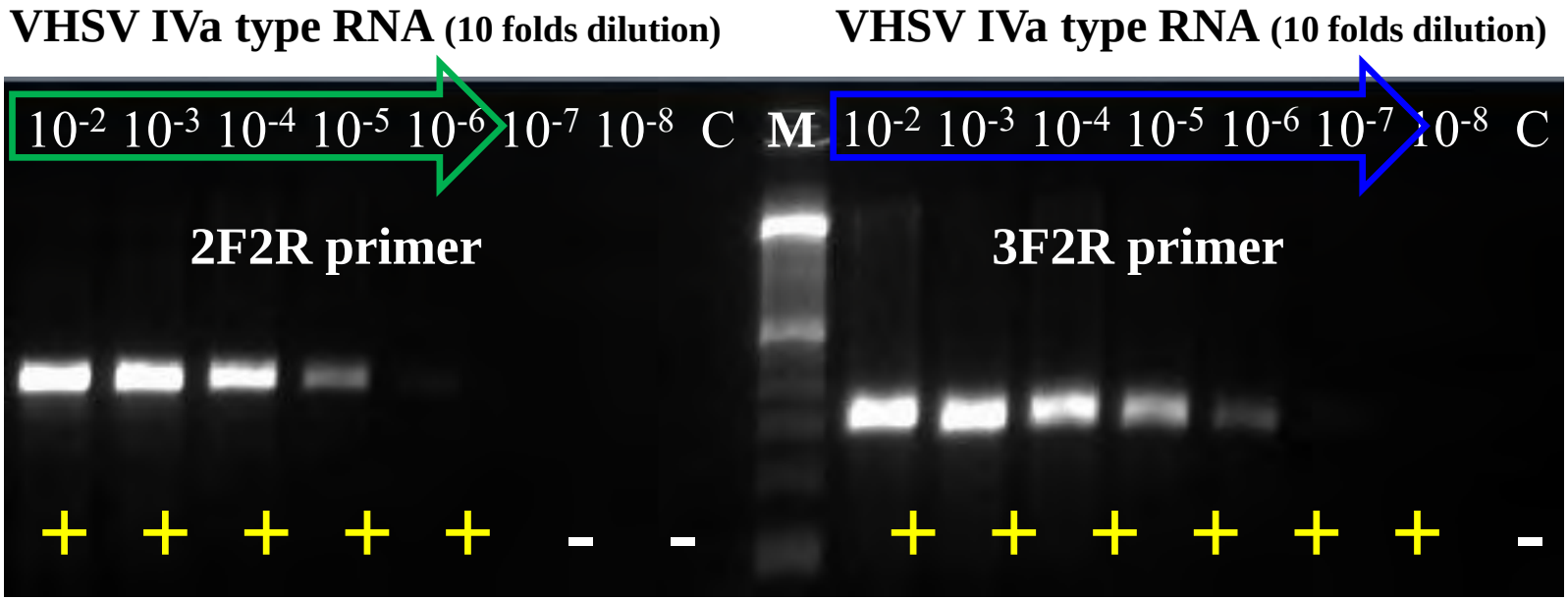


Materials and Methods

- Template : spleen from challenged flounder with VHSV KJ2008 (IVa)
- RNA extraction and RT-PCR
- PCR condition : OIE manual (VN primer set)

→ **2F2R & 3F2R primer sets amplified VHSV IVa at all temperatures.**

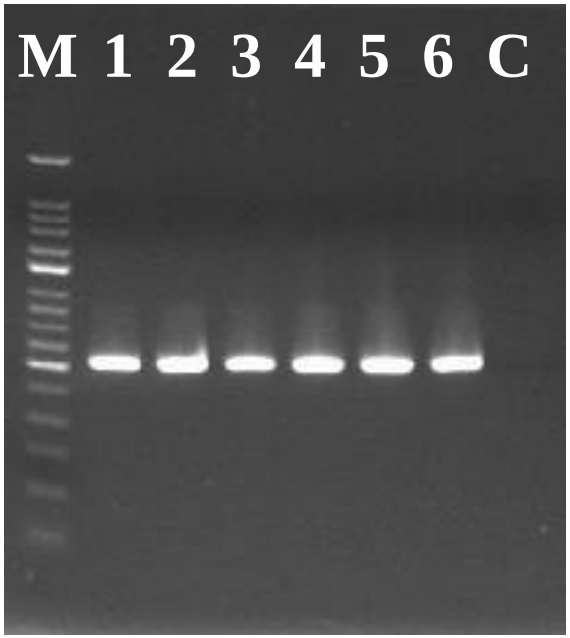
RT-PCR titration results for selection of one primer set



→ 3F2R primer set showed higher sensitivity than 2F2R.
So, the 3F2R primer set was selected.

Influence of 3F2R RT-PCR from various primer companies

Primer set from 6 companies tested :



M : 50 bp DNA size marker,
Lane 1-5 : each Korean companies
Lane 6 : Denmark company
C : Negative control

→ **No effects of different primer companies.**

The Specificity test of RT-PCR using 3F2R primer set on heterologous viruses

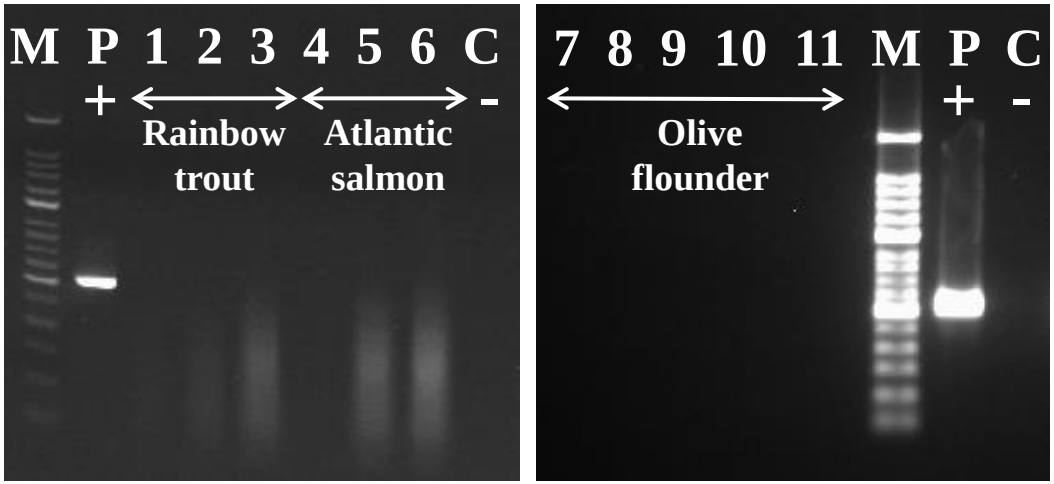
9 Heterologous viruses

Lane 1 : Positive control
Lane 2 : IHNV F-32/87
Lane 3 : IHNV I-4008
Lane 4 : IHNV DW
Lane 5 : IHNV BC
Lane 6 : Birnavirus II
Lane 7 : LGV
Lane 8 : PFR
Lane 9 : SVC 56/70 Fijan
Lane 10 : KHV H361
C : Negative control

→ **No bands showed on 9 heterologous virues.**

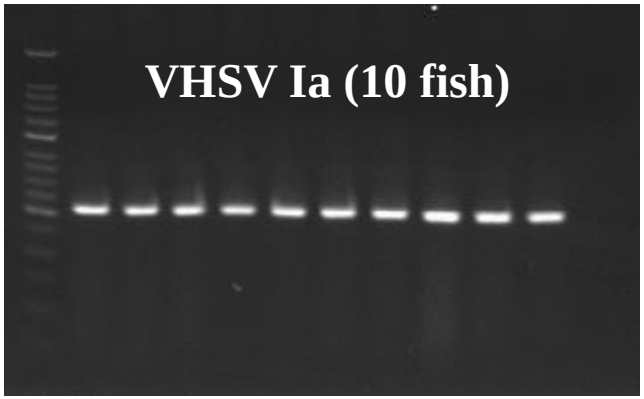
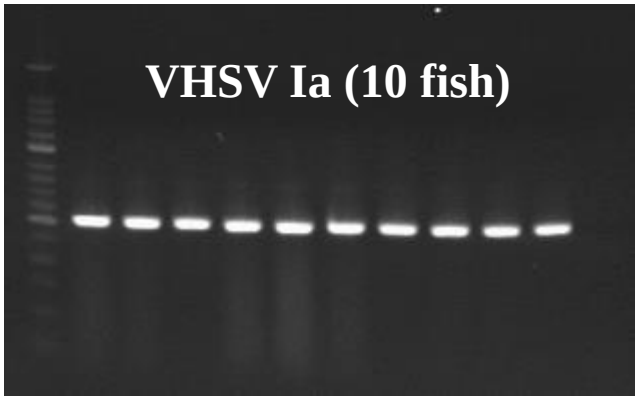
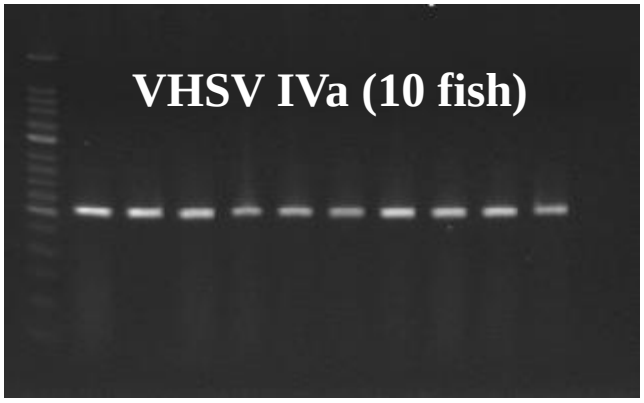
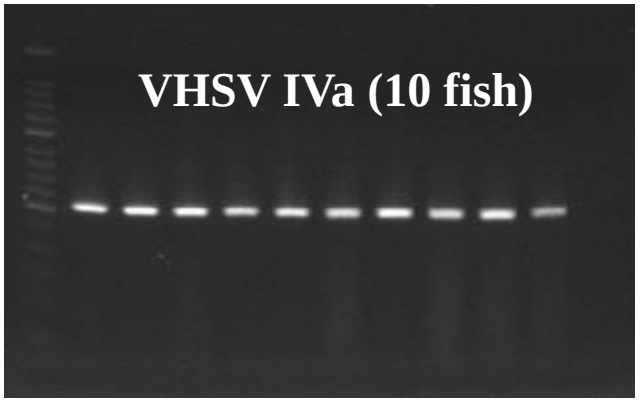
Non-specific reactions of RT-PCR using 3F2R primer set on tissue samples from normal rainbow trout, Atlantic salmon and olive flounder

Non-infected fish samles



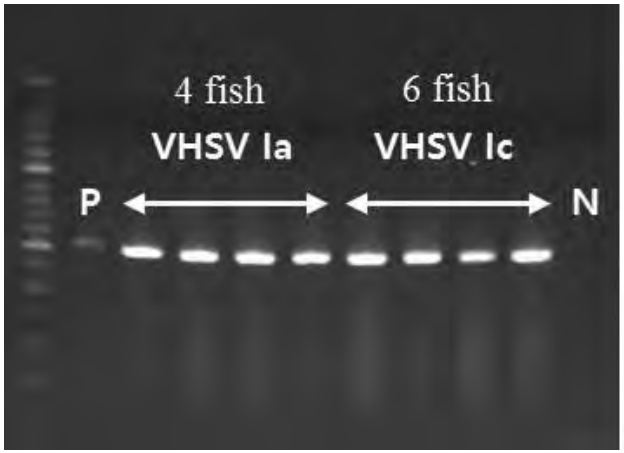
→ **No bands** showed in tissue samples from 3 fish species.

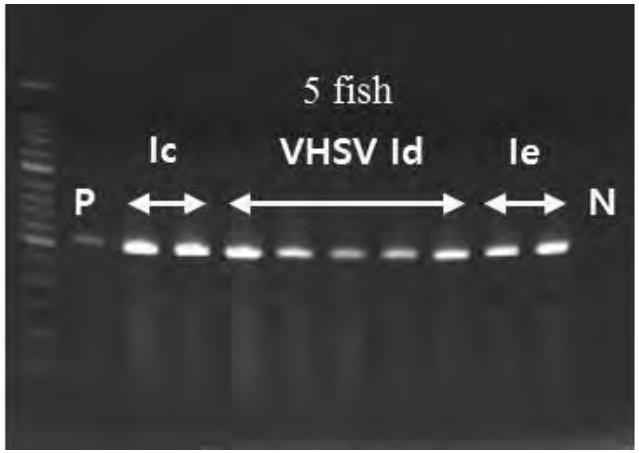
RT-PCR using 3F2R primer on samples from VHSV infected fish

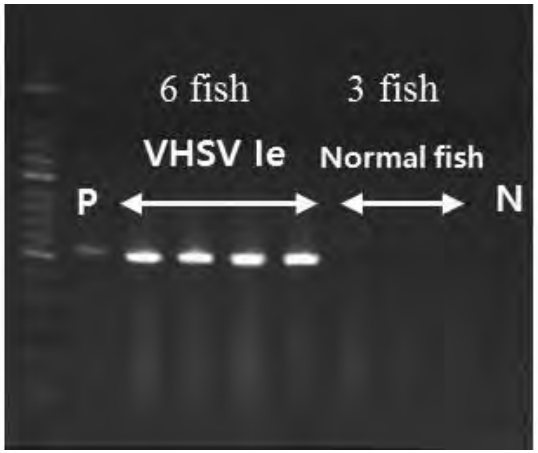
	Atlantic salmon IP infection	Rainbow trout bath infection
VHSV Ia challenged fish		
	Atlantic salmon IP infection	**Rainbow trout IP infection**
VHSV IVa challenged fish		

→It was confirmed that only specific bands were observed using the 3F2R primer set on VHSV fish infected samples.

RT-PCR using 3F2R primer on samples from VHSV I subtypes infected rainbow trout







→It was confirmed that only specific bands were observed using the 3F2R primer set on samples from rainbow trout infected with VHSV sub-type Ia, Ic, Id and Ie.

Summary 1

- **A highly sensitive primer set was selected** among several new candidate primers.

- **Reaction conditions were established** for this conventional RT-PCR without non-specific reactions in fish, fish cell lines or with heterologous viruses.

Comparison of sensitivities of several methods

- Real-time RT-PCR (Jonstrup et al. 2013)
- OIE conventional RT-PCR
- 3F2R conventional RT-PCR
- Cell cultures for virus titration(TCID50)

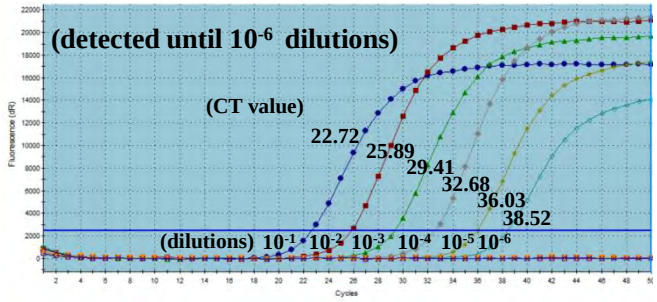
Selection of 6 VHSV isolates representing all major genotypes

Genotype	Isolate name	Source of isolate	Used cell lines
Ia	DK-3592B	Lorenzen et al. (1993)	BF-2
Ib	DK-1p8	Mortensen et al. (1999)	BF-2
II	DK-1p52	Mortensen et al. (1999)	FHM
III	DK-4p168	Mortensen et al. (1999)	EPC
IVa	KJ2008	Kim & Kim (2011)	EPC
IVb	MIO3, Lakes St. Clair, MI	Elsayed et al. (2006)	EPC

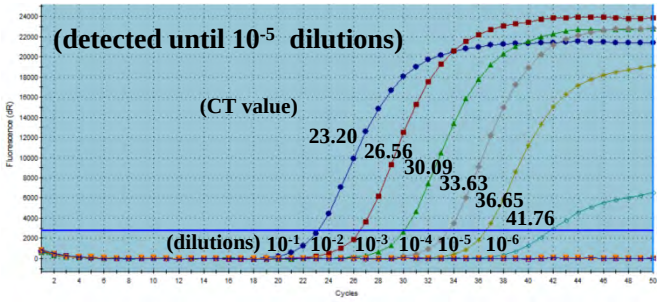
→ **The 6 VHSV isolates used for comparison of sensitivities using several detection methods.**

RT-qPCR titrations from 6 VHSV isolates representing all genotypes

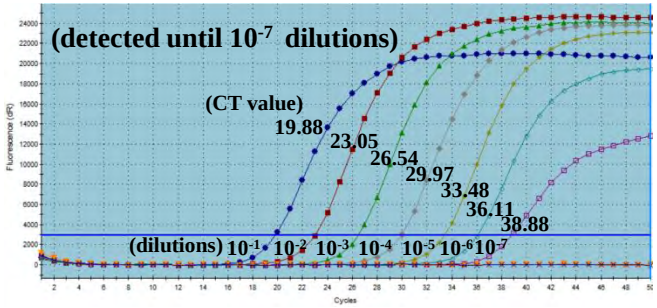
VHSV genotype Ia



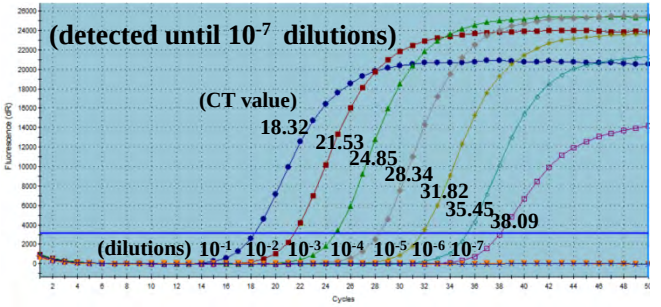
VHSV genotype Ib



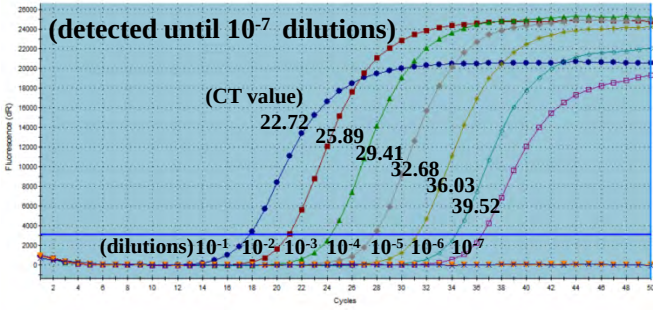
VHSV genotype II



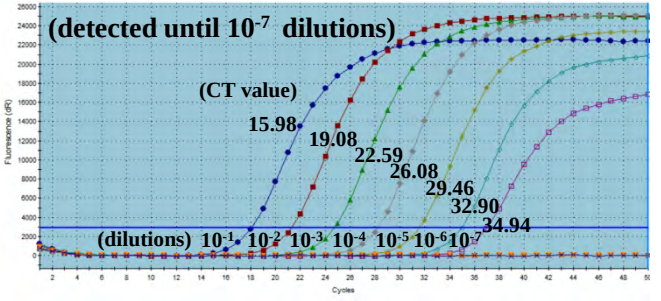
VHSV genotype III



VHSV genotype IVa



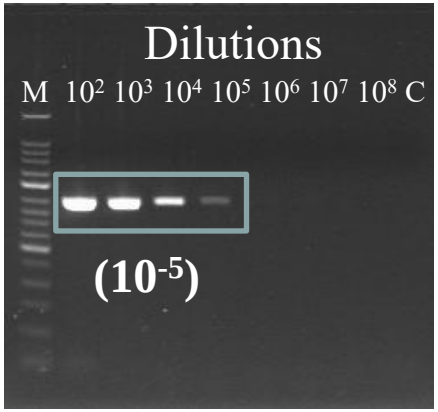
VHSV genotype IVb



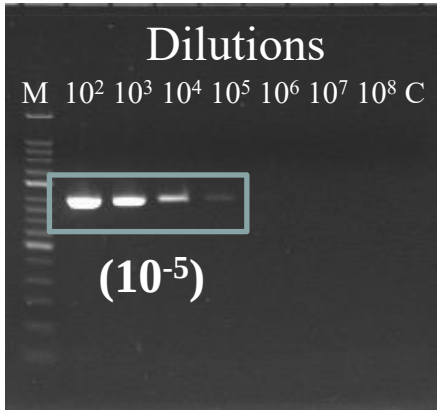
→ **In this results, the viral genes were detected at dilutions between 10^{-5} and 10^{-7}.**

OIE VN primer RT-PCR on titrations from 10^{-2} to 10^{-8} of 6 VHSV isolates representing all genotypes

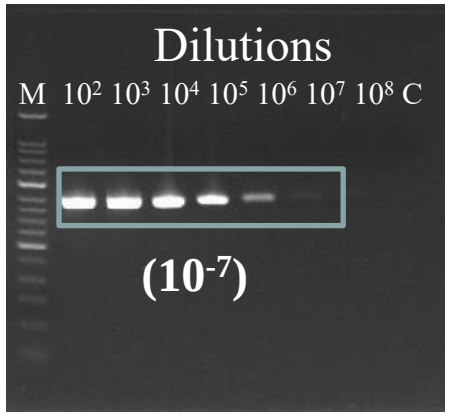
VHSV genotype Ia (10^{-5})



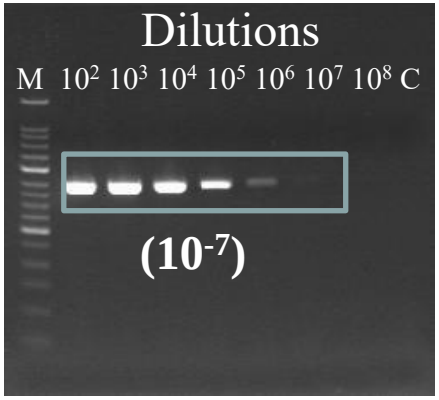
VHSV genotype Ib (10^{-5})



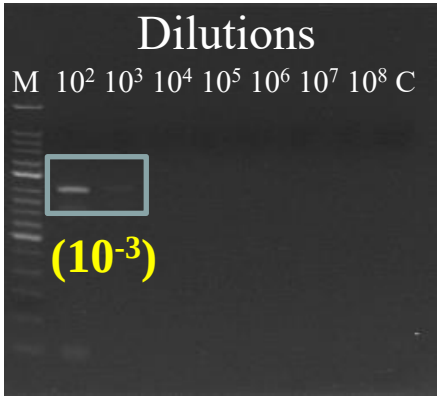
VHSV genotype II (10^{-7})



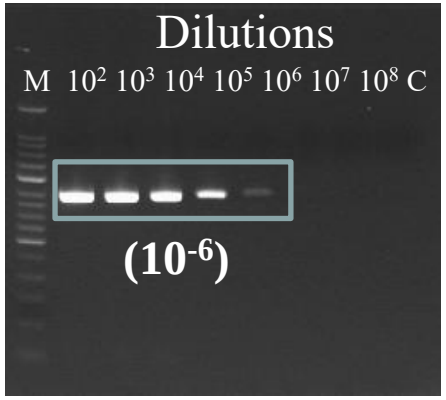
VHSV genotype III (10^{-7})



VHSV genotype IVa (10^{-3})



VHSV genotype IVb (10^{-6})

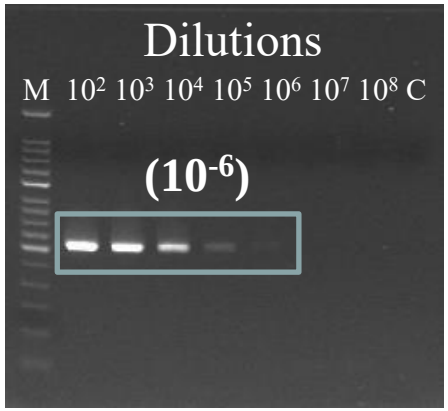


→ **The viral genes were detected at dilutions between 10^{-3} and 10^{-7}.**

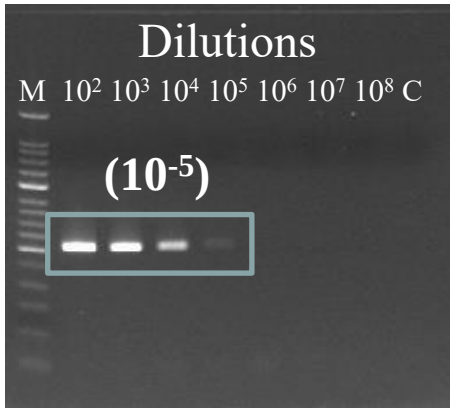
→ **The OIE VN primer only detected VHSV IVa at a very low level.**

3F2R primer RT-PCR on titrations from 10^{-2} to 10^{-8} of 6 VHSV isolates representing all genotypes

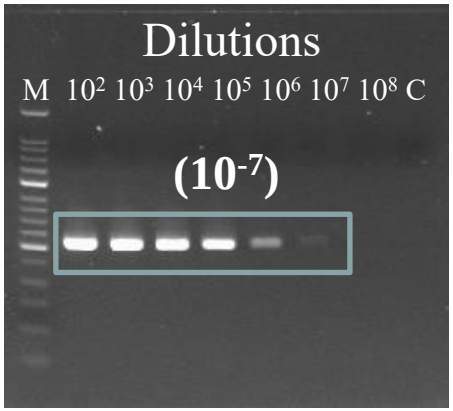
VHSV genotype Ia



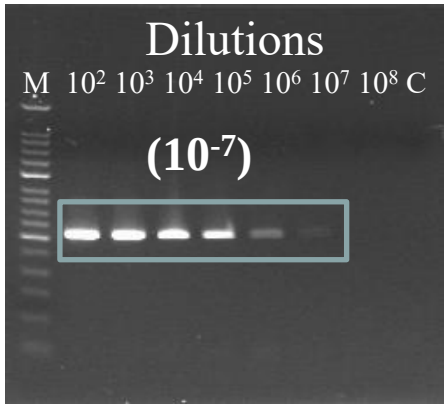
VHSV genotype Ib (10^{-5})



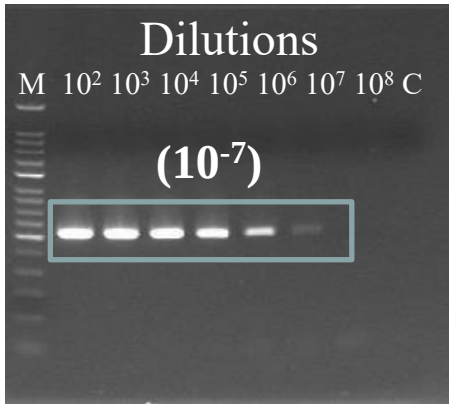
VHSV genotype II (10^{-7})



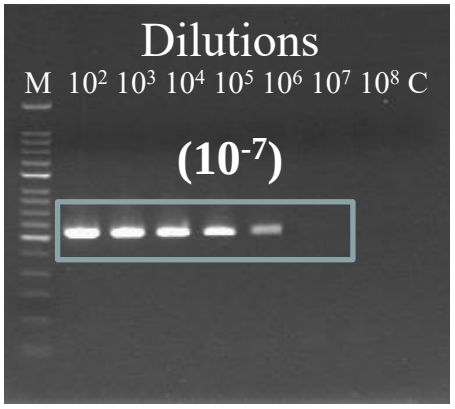
VHSV genotype III



VHSV genotype IVa (10^{-7})



VHSV genotype IVb (10^{-7})



→ **The viral genes were detected at dilutions between 10^{-5} and 10^{-7}.**

→ **The 3F2R primer set detected all VHSV at high level.**

Summary 2

Sensitivities of 3 RT-PCR and cell culture for detection of VHSV.

VHSV genotypes	Cell culture	Real-time RT-PCR	Conventional RT-PCR using OIE primer	Conventional RT-PCR using 3F2R primer
Ia	-6	-6	-5	-6
Ib	-5	-5	-5	-5
II	-7	-7	-7	-7
III	-7	-7	-7	-7
IVa	-7	-7	-3	-7
IVb	-7	-7	-6	-7

10,000 folds low →

← 10,000 folds low

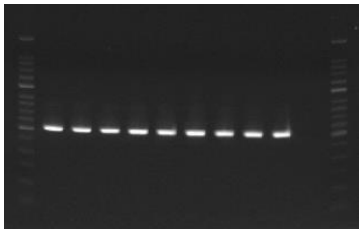
→ It was concluded that the sensitivity for all genotypes were at the same level when using cell culture, real-time RT-PCR and the conventional 3F2R RT-PCR. While it was lower for the OIE VN RT-PCR.

The 80 VHSV isolates for specificity test of 3F2R RT-PCR

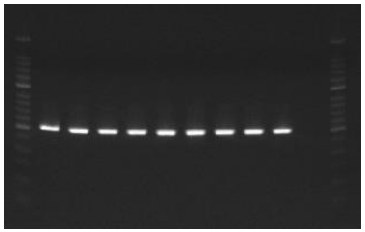
Genotypes and subtypes	Isolate numbers
I	1 - 2
Ia	3 - 18
Ib	19 - 32
Ic	33 - 35
Id	36 - 38
Ie	39 - 40
II	41 - 44
IIIa	45 - 54
IIIb	55
IVa	56 - 74
IVb	75 - 79
IVc	80

3F2R primer RT-PCR on 80 VHSV isolates

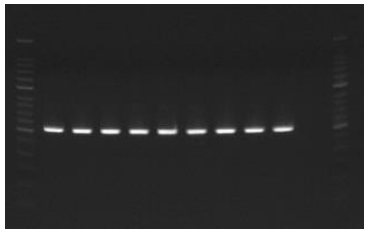
Isolate num. 1 - 9



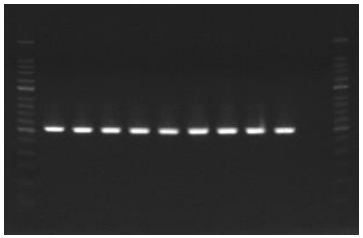
Isolate num. 10 - 18



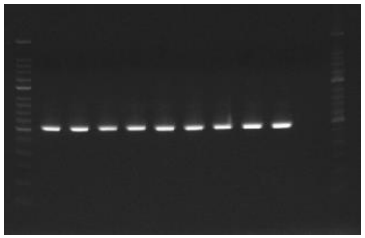
Isolate num. 19 - 27



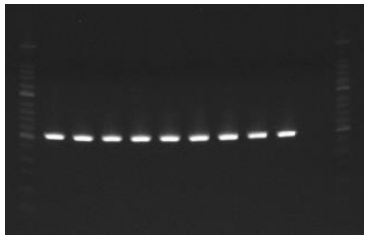
Isolate num. 28 - 36



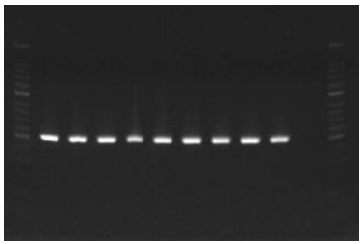
Isolate num. 37 - 45



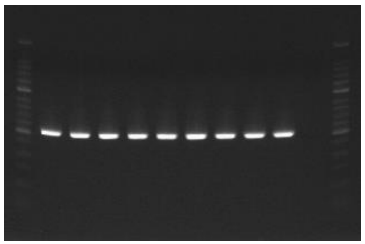
Isolate num. 46 - 54



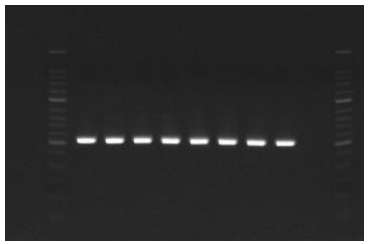
Isolate num. 55 - 63



Isolate num. 64 - 72

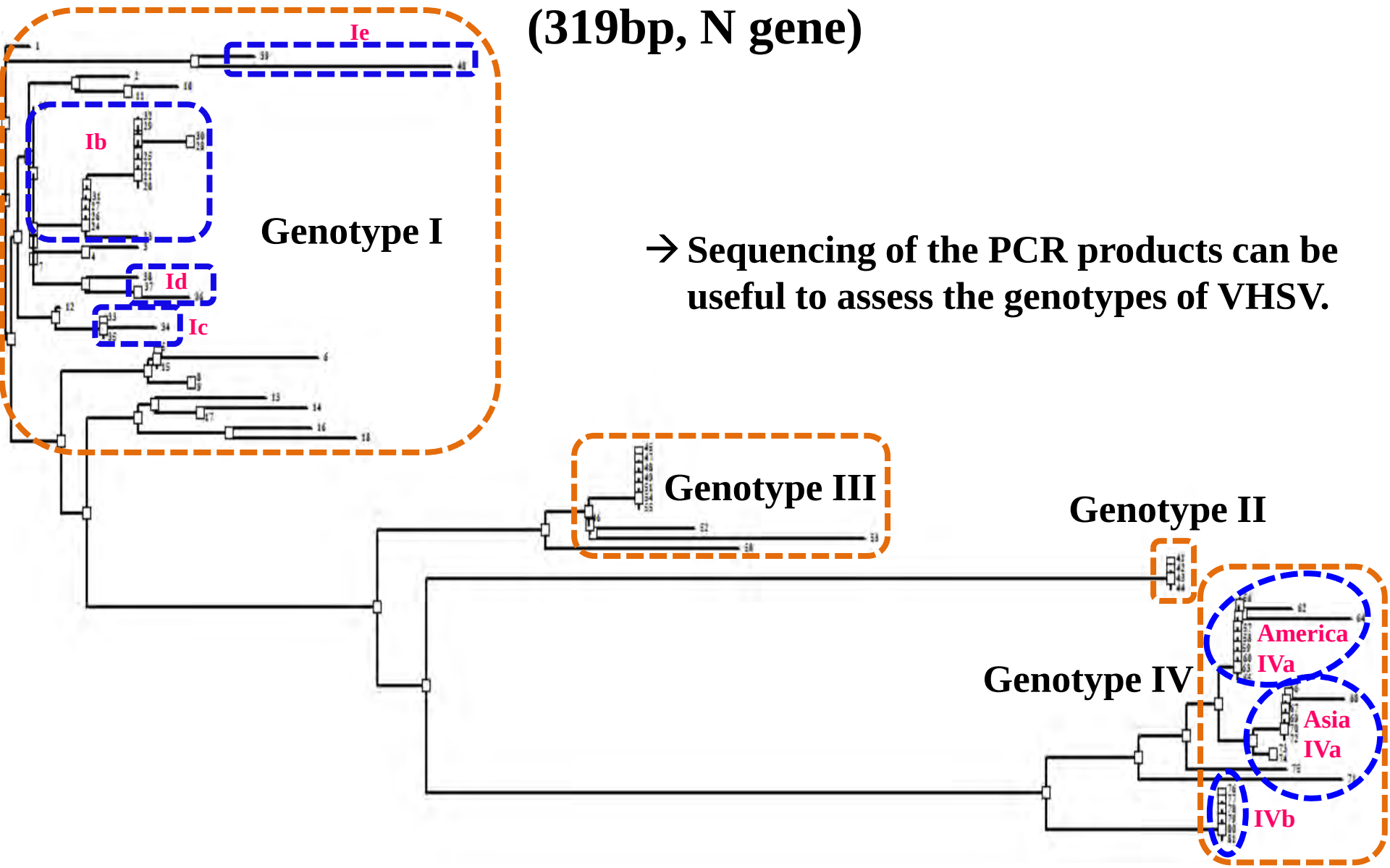


Isolate num. 73 - 80



→ **Clear and unique amplicons were observed for all 80 VHSV isolates representing a worldwide collection of all known genotypes and subtypes.**

Phylogenetic analysis of all amplicons from 80 VHSV isolates (319bp, N gene)



→ Sequencing of the PCR products can be useful to assess the genotypes of VHSV.

Inter-laboratory proficiency test (PT) of 3F2R RT-PCR

- **To assess the reproducibility and robustness of the 3F2R conventional RT-PCR by an inter-laboratory proficiency test among 9 selected laboratories were conducted.**

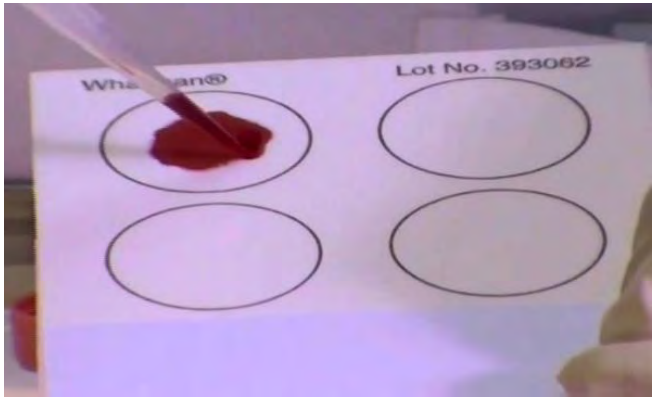
[Italy (IZSVe), France (ANSES), UK (CEFAS), Germany (FLI, two laboratory), Denmark (DTU), Japan (NRIA, two laboratory), Korea (NFQS)]

Sample preparation (10) on FTA cards

- 6 VHSV samples : VHSV I, Ib, II, III, IVa, IVb
- 3 heterologous virus : IPNV, HRV, IHNV
- 1 control : only normal cell culture medium

The viral supernatants were dropped on FTA cards (Whatmann Company).

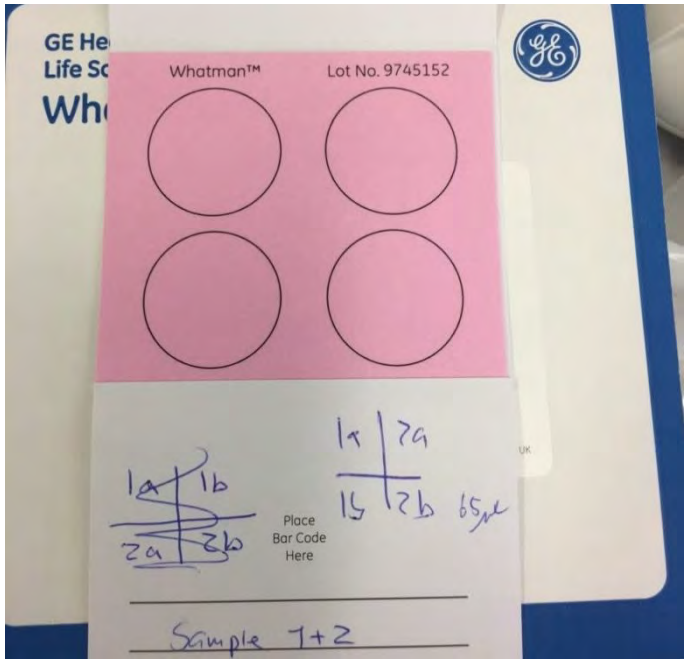
What is FTA cards ?



Chemical formula on the cards
- lysis cell membrane and denature protein on contact
- Nucleic acids : entrapped, immobilised and stabilised

Advantage of FTA cards
- protect nucleic acids from nucleases, oxidation, UV damage and microbial and fungal attack
- inactivation: infectious pathogens
- stable for storage at room temperature

Preparation of FTA cards for inter laboratory Proficiency test



Put on FTA cards (10 samples)

Packaging the 5 cards (1-10 samples)

SOP for 3F2R

SOP for detection of VHSV by the "3F2R" conventional RT-PCR

AIM

To assess the reproducibility of a novel conventional RT-PCR for detection of viral hemorrhagic septicemia virus (VHSV) by an inter-laboratory proficiency test among 6 selected laboratories using the Kim3F2R primer set.

BACKGROUND

Conventional RT-PCR is typically used for detecting VHSV and for genotyping the virus. However, using the primers and procedures given in the VHSV chapter of the OIE Aquatic Manual we found a low sensitivity for detection of VHSV IVa isolates. In addition, non-specific reaction with fish cell lines was often observed when using the OIE RT-PCR. Thus, there was a need for improvement of the VHSV conventional RT-PCR given in the OIE Diagnostic Manual with regard to specificity and sensitivity in order to detect all VHSV genotypes and to remove the non-specific reactions due to fish cell lines.

Candidate primers from 5 regions of the VHSV nucleoprotein (N) gene were tested, and a highly sensitive primer set (Kim3F2R) was selected among these. The reaction conditions of the selected primer set were established and no non-specific reactions in fish, fish cell lines or with heterologous viruses were observed. The sensitivity of new RT-PCR was tested in parallel with cell cultivation, the "Jonstrup et al." RT-qPCR, and the conventional OIE VN RT-PCR. It was concluded that the sensitivity for all VHSV genotypes wasere at the same level when using cell culture, qPCR, and the new conventional RT-PCR except for conventional OIE VN RT-PCR. The novel RT-PCR was following tested on 80 VHSV isolates representing a worldwide collection of all known genotype and subtypes, where it produced clear and unique amplicons for all 80 isolates.

REAGENTS

1) Isolation of RNA

   Qiagen RNeasy minikit from Qiagen, 70% ethanol, 2-mercaptoethanol

2) New conventional RT-PCR

   Qiagen Onestep RT-PCR kit, Forward primer (VHSV 3F), Reverse primer (VHSV 2R), Takara 50bp marker, loading dye, agarose gel

   ☞ Primer sequence : VHSV 3F 5' - GGG-ACA-GGA-ATG-ACC-ATG-AT - 3',

   VHSV 2R 5'- TCT-GTC-ACC-TTG-ATC-CCC-TCC-AG - 3'

METHODS

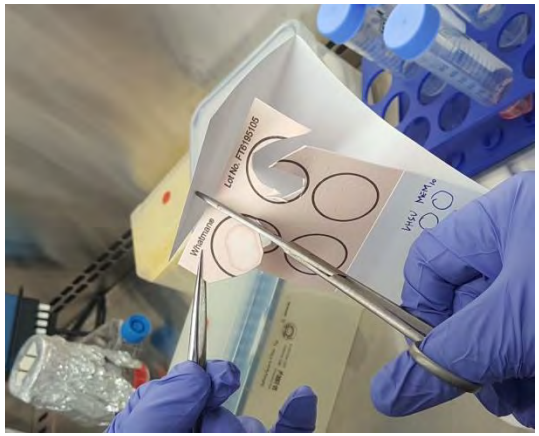
All procedures should be carried out on ice or in a cooler in a laminar airflow cabinet.

RNA EXTRACTION from FTA cards

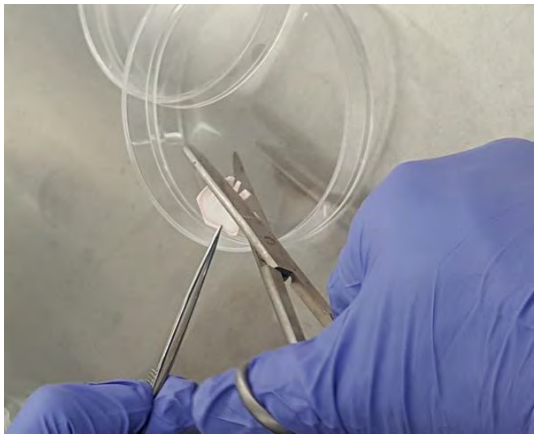
1. For the RNA extraction, all work should be performed on ice, using gloves.
2. With help of scalpel blade or scissors cut out a small piece (approximately 0.5 cm in diameter) from the area where the sample has been adsorbed (within the large circle drawn on the card) and place it in a 1.5 mL tube
3. Add 500 µl RLT buffer (lysis buffer) and 5 µl of 2-mercaptoethanol in the tube* and mix thoroughly by pipetting up and down at least 5 times. Hereafter place the tube on a tilt table for one hour at room temperature.

→We sent the SOP and FTA cards for assesment of 3F2R primer RT-PCR to selected 9 laboratories.

Analysis methods



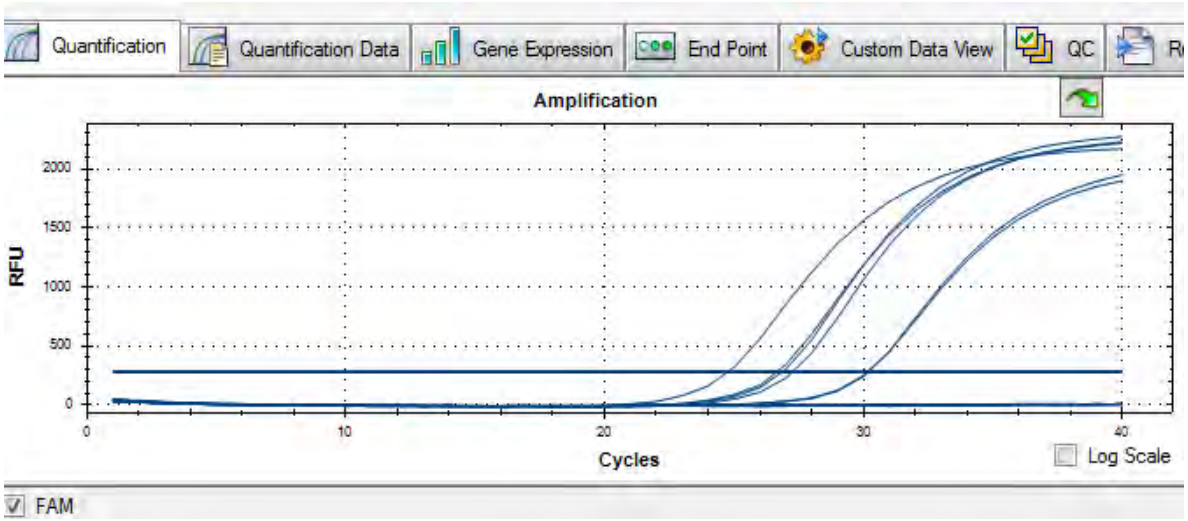
Cutting of FTA cards



Cut into small pieces and mixed lysis buffer

- Elution and RNA extraction from the FTA cards
- Real-time RT-PCR for VHSV detection
- RT-PCR using VN (OIE) primer set for VHSV detection
- RT-PCR using 3F2R primer set for VHSV detection

qRT-PCR results using Jonstrup et al. method

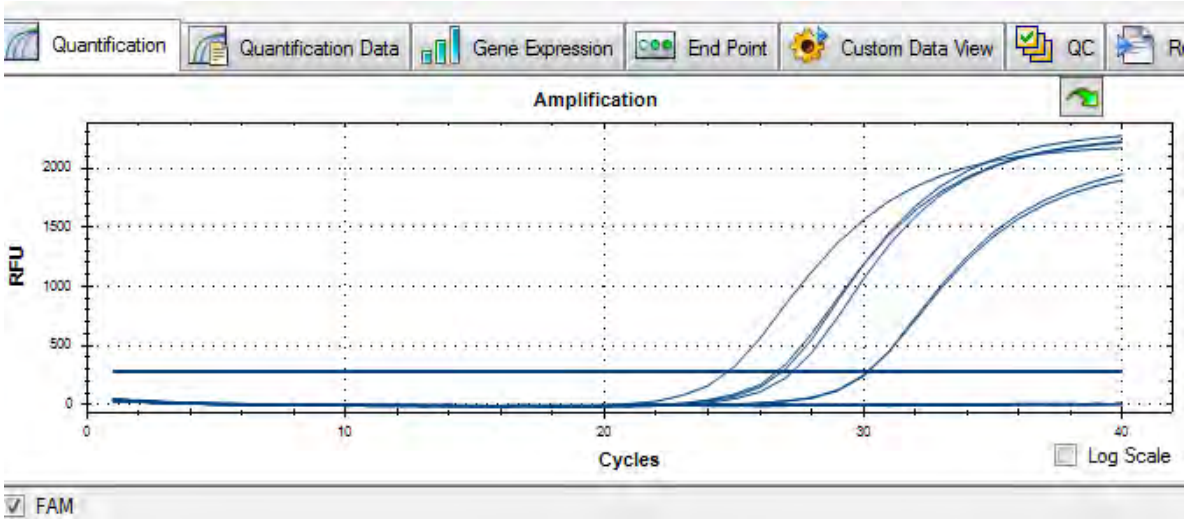


Sample	Cq
FTA 1	24,72
FTA 2	N/A
FTA 3	30,17
FTA 4	N/A
FTA 5	26,82
FTA 6	27,22
FTA 7	N/A
FTA 8	26,61
FTA 9	N/A
FTA 10	30,13

CT value	Sample	Isolate	VHSV genotypes
	S 1	DK-F1	**Genotype I**
	S 2	IPN SP	
	S 3	DK-1p52	**Genotype II**
	S 4	HRV8401	
	S 5	Goby 1-5	**Genotype IVb**
	S 6	JF-JF00Ehi	**Genotype IVa**
	S 7	IHN 32/87	
	S 8	DK-4p168	**Genotype III**
	S 9	Medium (cell control BF-2)	
	S10	DK-1p8	**Genotype Ib**

Positive results : Sample 1, 3, 5, 6, 8, 10

qRT-PCR results using Jonstrup et al. method



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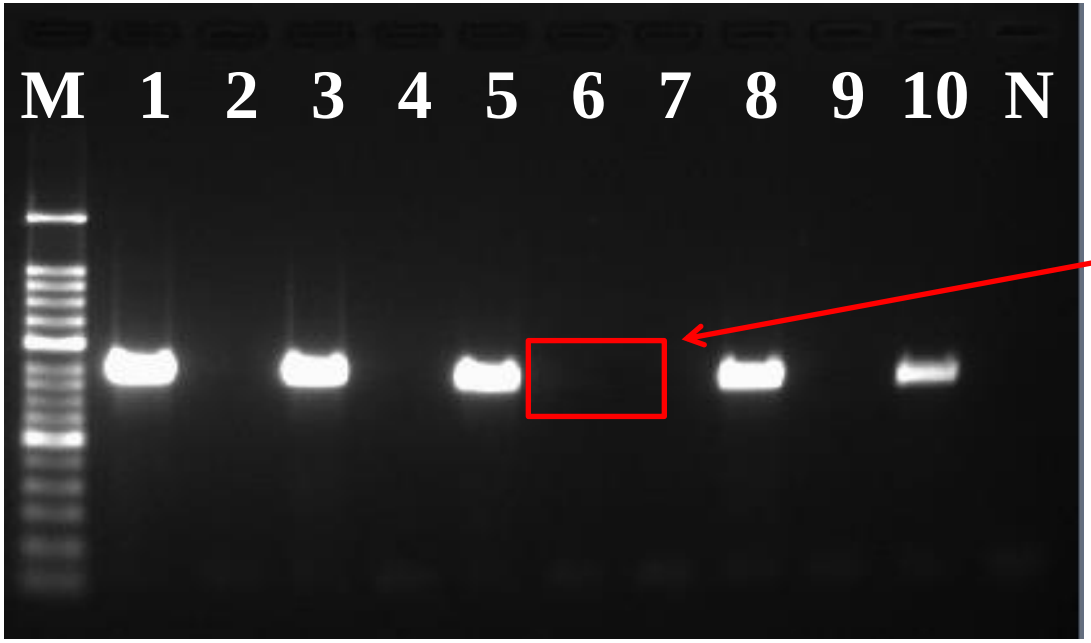
	S 6	JF-JF00Ehi	**Genotype IVa**
	S 7	IHN 32/87	
	S 8	DK-4p168	**Genotype III**
	S 9	Medium (cell control BF-2)	
	S10	DK-1p8	**Genotype Ib**

Almost same level of viral RNA : 5, 6, 8

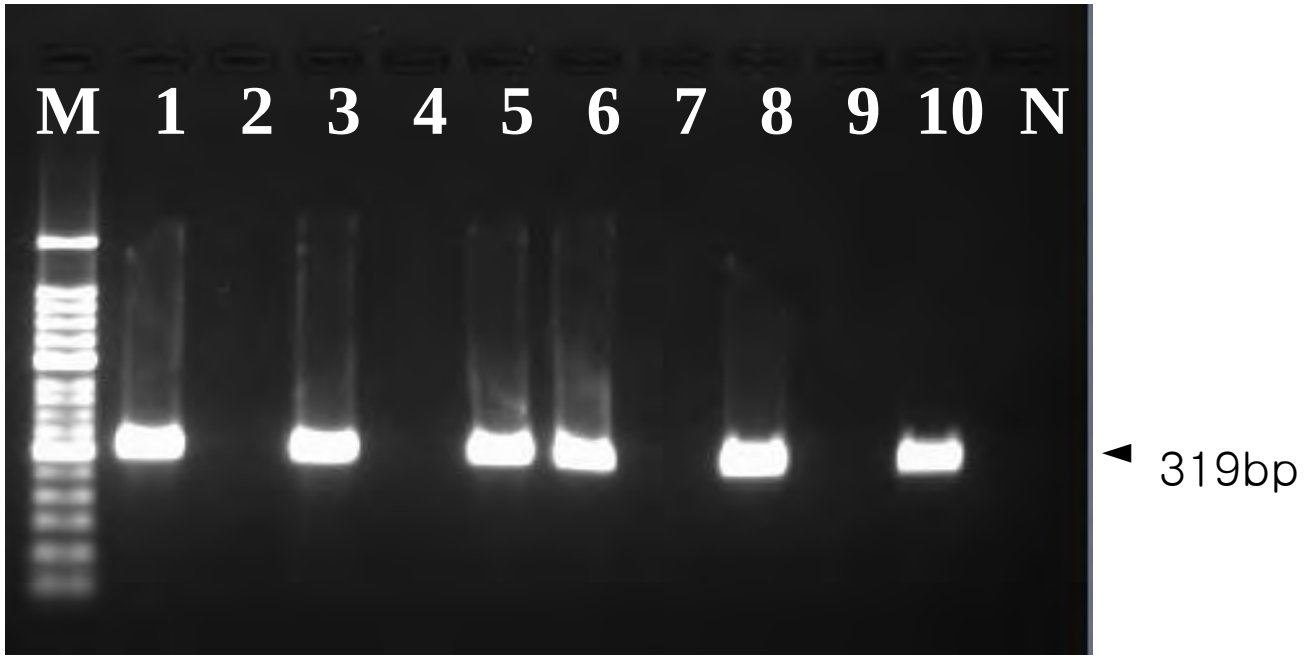
Conventional RT-PCR results using OIE VN primer



M : 50 bp DNA marker
1. DK-F1(Genotype I) 2. IPN SP 3. DK-1p52 (Genotype II) 4. HRV8401
5. Goby 1-5(Genotype IVb) 6. JF-JF00Ehi(Genotype IVa) 7. IHN 32/87
8. DK-4p168(Genotype III) 9. Medium (cell control BF-2)
10. DK-1p8(Genotype Ib)

Positive results : Sample 1, 3, 5, ~~6~~, 8, 10

Conventional RT-PCR results using 3F2R primer



M : 50 bp DNA marker
1. DK-F1(**Genotype I**) 2. IPN SP 3. DK-1p52 (**Genotype II**) 4. HRV8401
5. Goby 1-5(**Genotype IVb**) 6. JF-JF00Ehi(**Genotype IVa**) 7. IHN 32/87
8. DK-4p168(**Genotype III**) 9. Medium (cell control BF-2)
10. DK-1p8(**Genotype Ib**)

Positive results : Sample 1, 3, 5, 6, 8, 10

Summary of the PCR results from 9 laboratories

Lab. Number	3F2R Conventional PCR	qPCR or Sequencing (option)
1	**Success** (Macherey Nagel Nucleospin Virus & Invitrogen superscript III one-step RT-PCR)	**Success** (qPCR, Jonstrup et al method) (Invitrogen superscript III one-step qRT-PCR)
2	**Success** (Qiagen Rneasy Mini Kit & Qiagen Onestep RT-PCR Kit)	**Success** (qPCR, Jonstrup et al method) (Qiagen QuantiTect RT Kit)
3	**Success** (QIAamp Viral RNA mini kit & Qiagen Onestep RT-PCR Kit)	**Success** (Sequencing and genotyping)
4	**Fail** (EZ-1 RNA tissue mini kit & EZ-1 BioRobot & Two step RT-PCR using MMLV and Go-Taq)	ND

One laboratory did not detected all VHSV isolates, it seems that the RNA extraction from FTA cards was not conducted smoothly.

Summary of the PCR results from 9 laboratories

Lab. Number	3F2R Conventional PCR	qPCR or Sequencing (option)
5	**Success** (QIAamp Viral RNA mini kit & Qiagen Onestep RT-PCR Kit)	ND
6	**Success** (Macherey Nagel Nucleospin Virus & Qiagen Onestep RT-PCR Kit)	**Success** (qPCR, Jonstrup et al method) (Qiagen QuantiTect RT Kit)
7	**Success** (Qiagen Rneasy Mini Kit & Qiagen Onestep RT-PCR Kit)	**Success** (qPCR, Jonstrup et al method) (Qiagen QuantiTect RT Kit)
8	**Success** (Qiagen Rneasy Mini Kit & Invitrogen superscript III one-step RT-PCR)	ND
9	**Success** (Qiagen Rneasy Mini Kit & Invitrogen superscript III one-step RT-PCR)	ND

However, other 8 laboratories were sucessfully confirmed the reproducibility of 3F2R primer set.

Conclusions

- **Specificity of the novel 3F2R method** was confirmed on organ materials from fish samples and large numbers of viruses.
- The **reproducibility and robustness of 3F2R method** were confirmed by 8 of 9 laboratories.
- Finally, **we suggest that the 3F2R primer set shall replace the current primer set recommended in the OIE manual for detection of VHSV by conventional RT-PCR**.


Thank You !

Thank you !!