Final Report of the OIE Twinning Project on Viral Haemorrhagic Septicaemia (VHS)

April 2018

National Veterinary Institute in Denmark

and

National Fishery Products Quality Management Service in Korea

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1. Summary of the project aims and objectives set out at the start, including the justification for the project

The National Fishery Products Quality Management Service (NFQS) is the competent authority in Korea responsible for quarantine of exported and imported fisheries goods according to the Aquatic Creature Disease Control Act, and is a state designated national Reference Laboratory (NRL). Viral Haemorrhagic Septicaemia (VHS) is a disease widespread around the world, and in Korea infection with VHSV genotype IVa has in particular been an issue each year. As the competent authority with regards to exports and imports of aquatic life, the NFQS has the duty to prevent other types of VHSV from entering Korea from other countries while at the same time ensuring that the VHSV IVa type does not spread to other countries as well. As a result, with diagnosis and prevention of VHS outbreak being so imperative in Korea, it was agreed by the two countries, organizations, and representatives in Korea and Denmark that NFQS would receive scientific assistance on how to systematically control VHS from the National Veterinary Institute (NVI) at the Technical University of Denmark (DTU) in Denmark, which is designated as an OIE Reference Laboratory for VHS, to better control the disease. In addition, both parties promised to cooperate towards the goal of NFQS being approved as an OIE Reference Laboratory on VHS in Asia. This project has already been launched with the approval of Danish and Korean CVOs, and on May 2015, it was officially announced at the OIE General Session.

After the official approval from the OIE, a kick-off meeting was held at the National Veterinary Institute in Denmark by project leads Prof. Niels Jørgen Olesen from Denmark and Dr. Hyoung Jun Kim from Korea in July 2015. And, we discussed several objectives to improve the diagnostic capacities, the role of the NFQS as an NRL, epidemiological tools and scientific relationships within the Asian region and to provide a technical support to any countries for VHS diagnosis and control.

The more detail objectives indicates below;

A. To improve the NFQS' capabilities to diagnose VHS

- Training courses at the parent laboratory

- Training on site at the premises of the candidate laboratory
- Proficiency testing
- Implementation of immunochemical and molecular methods
- Practical and theoretical courses at the parent and candidate institutes
- Proficiency test shipped and performed

B. To strengthen capabilities as an NRL and to develop diagnostic methods

- Collection and characterization of VHSV isolates present in Asia
- Collection of VHSV isolated in Korea and research on its genes
- Phylogenetic analysis of VHSV genes
- Comparison and study of pathogenicity of viruses with phylogenetic differences
- Evaluation of VHS detection methods using conventional RT-PCR
- Development of VHS detection method using digital RT-PCR capable of absolute quantification
- Development of methods to prevent misdiagnosis caused by contaminated target genes in real-time RT-PCR

C. To enhance the credibility of test results by obtaining ISO 17025 accreditation on VHS

- Accreditation of NFQS labs with ISO 17025

D. To establish virus management system including VHSV collection

- In collaboration with other scientists in Korea to establish and manage a repository of VHSV and their genetic characteristics

2. Description of situation in Candidate Centre at the beginning of the project and the priority areas that were selected for improvement

2.1. Needed accreditation of ISO17025 for VHS diagnosis at NFQS laboratory

NFQS had a high containment laboratory facility for VHS and other diseases at the beginning of the project. The rooms of the laboratory were divided into cleaning and sterilization, reagent and materials, autopsy, histopathology, cell culture for normal and inoculated cells (BSL3), extraction of nucleic acids, master mix room for PCR, mix the nucleic acids in the PCR cocktail, positive control, PCR machine, real-time PCR machine, and electrophoresis. The laboratory had been managed according to the OIE Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases. But, laboratory of NFQS not prepared the accreditation of ISO17025 for VHS diagnosis at the beginning of the project.

2.2. Leak of concept about validation of diagnostic tools including serological diagnostic tools for VHS diagnosis in the OIE

NFQS had many experiences for gene detection and cell culture of infectious agents in aquatic animal field. Specially, Dr. Kim and his team had developed a chimeric PCR positive plasmid control without the use of pathogen nucleic acids or pathogen-infected tissues for prevention of gene contamination from PCR positive DNA. In addition, NFQS had completely prepared molecular biological methods including two methods (Jostrup et al. 2013 and Garver et al., 2011) of real time RT-PCR in OIE manual of diagnostic test for VHS. But, laboratory of NFQS not prepared serological methods including neutralization, ELISA and IFAT for VHS diagnosis in the OIE manual of diagnostic test.

2.3. Leak of activities of international cooperation, presentation of research results and education

Dr. Kim obtained a Doctor of Philosophy in the field of Marine Life Science, Division of Marine Life Science in the graduate School of Fisheries Sciences at Hokkaido University, Hakkodate, Japan, in 2009. The University is focused on research of salmonid diseases and was appointed the OIE Reference Laboratory for *Oncorhynchus masou* virus disease

(OMVD). And he has a good relationship with experts of OIE Reference Laboratory on KHV and RSIV in Japan by attending their training courses, various meeting and cooperating in research. In 2011, he was delegated as a specialist by the Korean government for detection of white spot syndrome virus (WSSV) from aquatic animals in China. He collaborated with a Chinese specialist on implementing diagnostic methods at the Sandong Entry-exit Inspection and Quarantine Bureau of China. Based on the experience, he has strengthened a great relationship with many experts in neighboring countries, but little relationship with English-speaking countries.

3. Any changes that were made to the initial project plan, such as a change in direction or scope

When the proposal of the OIE Twinning project was presented to the OIE Aquatic Animal Health Standards Commission, the commission suggested that the objective of this twining project should concentrate on increasing the diagnostic capacities of the candidate laboratory, whereas the Twinning project should not aim to become a research project to characterize the VHS virus isolates in the region. We changed the original plans based on these comments from OIE Aquatic Animal Health Standards Commission. It was scientifically proven by Dr. Kim that the detection of VHSV genotype IVa by using conventional reverse-transcription PCR method results in low sensitivity. Thus, we changed the original plans to concentrate more to develop a novel conventional RT-PCR for detection of VHSV all genotypes according to the OIE guidelines for validation of new diagnostic methods without doing research on the characterization of VHSV isolates in Korea.

4. Description of activities including publications and presentations, training and consultation experience (courses provided, number of people trained, examples of international consultation), scientific meetings, contribution to the reference document “Level of diagnosis test manual on Aquatic animal disease

4.1 The list of publications during OIE Twinning project comprise five papers in peer-reviewed international journals, two patents for diagnostic tools, and multiple presentations and abstracts at international conferences and meetings.

- **Publications**

1) 2018. **H. J. Kim**, A. Cuenca, **N. J. Olesen**. Validation of a novel one-step reverse transcription PCR for detection of viral haemorrhagic septicaemia virus. Aquaculture, 492:170-183

2) 2018. J. S. Lee, J. Kim, S. R. Im, S. W. Kim, J. M. S. Lazarte, J. W. Jung, T. W. Gong, Y. R. Kim, J. H. Lee, **H. J. Kim**, T. S. Jung. Generation and characterization of hagfish variable lymphocyte receptor B against glycoprotein of viral hemorrhagic septicemia virus (VHSV). Molecular Immunology, 99: 30-38

3) 2016. **H. J. Kim**, J. S. Park, M. C. Choi, S. R. Kwon. Comparison of the efficacy of Poly (I:C) immunization with live vaccine and formalin-killed vaccine against viral hemorrhagic septicemia virus (VHSV) in olive flounder (*Paralichthys olivaceus*). Fish & Shellfish Immunology, 48: 206-211

4) 2015. **H. J. Kim**, K. H. Kim, J. S. Park, H. L. Lee, H. C. Kwon, S. R. Kwon. Immunological significance of recombinant VP2 and VP3 proteins of aquabirnavirus in olive flounder, *Paralichthys olivaceus*. Journal of Fish Pathology, 28: 93-98

5) 2015. **H. J. Kim**. 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. Aquaculture, 448: 359-364

6) 2015. **H. J. Kim**, J. S. Park, S. R. Kwon. Development of a stringent ELISA protocol to evaluate anti-viral hemorrhagic septicemia virus-specific antibodies in olive flounder *Paralichthys olivaceus* with improved specificity. Journal of Microbiology, 53: 481-485

- **Book**

1) S. W. Park, **H. J. Kim**. 2016. Fish Disease in Recent Outbreaks (in Korean). ISBN: 9788968240577

- **Oral Presentations**

1) **H. J. Kim**, **N. J. Olesen**. 2017. Outcome of the OIE Twinning Project between Korea and Denmark. Final meeting of OIE Twinning Project; 2017 December 11; Lyngby, Denmark

2) **H. J. Kim**, J. S. Park, J. H. Kim, S. Y. Kim, H. D. Song, S. W. Jeon, S. R. Kwon. 2017. Comparative expression profiling of immune-related genes in olive flounder,

Paralichthys olivaceus after vaccination with Poly(I:C)-VHSV of formalin-killed VHSV using next generation sequencing. Spring Meeting of the Korean Society of Fish Pathology, Jeju in Korea

3) **H. J. Kim**, A. Cuanca, **N. J. Olesen**. 2017. Development and validation of a novel one-step reverse transcription PCR method for detecting viral haemorrhagic septicaemia virus. The 18th International Conference on Diseases of Fish and Shellfish; 2017 September 4-8; Belfast, UK.

4) **H. J. Kim**, A. Cuanca, **N. J. Olesen**. 2017. Development and validation of a novel one-step reverse transcription PCR method for detecting viral haemorrhagic septicaemia virus. The 18th International Conference on Diseases of Fish and Shellfish; 2017 September 4-8; Belfast, UK.

5) **H. J. Kim**, K. Yuasa. 2017. Comparison of susceptibility of koi herpesvirus (KHV) between koi carp, *Cyprinus carpio* and ginbuna, *Carassius auratus langsdorfii*. The 18th International Conference on Diseases of Fish and Shellfish; 2017 September 4-8; Belfast, UK.

6) **H. J. Kim**, S. R. Kwon, K. Yuasa. 2017. Detection of viral DNA, mRNA and infectivity in koi fin (KF-1) cells infected with different concentration of koi herpesvirus (KHV). The 18th International Conference on Diseases of Fish and Shellfish; 2017 September 4-8; Belfast, UK.

7) **H. J. Kim**, **N. J. Olesen**. 2017. The status of the OIE Laboratory Twinning project for Viral Haemorrhagic Septicaemia (VHS) between Korea and Denmark. International Expert Workshop, Twinning Programme 2017 [Recent status and control of koi herpesvirus (CyHV-3) in South-East Asians]; 2017 August 26-27; Bali, Indonesia.

8) **H. J. Kim**, S. R. Kwon, K. Yuasa. 2017. The status of the koi herpesvirus in Korea. International Expert Workshop, Twinning programme 2017 [Resent status and control of koi herpesvirus (CyHV-3) in South-East Asians]; 2017August 26-27; Bali, Indonesia.

9) **H. J. Kim**, **N. J. Olesen**. 2017. Validation of Viral Haemorrhagic Septicaemia (VHS) virus conventional RT-PCR. 21th Annual Workshop of the EU National Reference Laboratories for Fish Diseases; 2017 May 31-31; Lyngby, Denmark.

10) **H. J. Kim**, S. S. Mikkelsen, **N. J. Olesen**. 2017. Development of a novel one-step reverse transcription PCR method for detecting viral haemorrhagic septicaemia virus. 10th International Symposium on Viruses of Lower Vertebrates; 2017 June 4-7; Budapest, Hungary.

11) **H. J. Kim**, **N. J. Olesen**. 2017. The status of the OIE Laboratory Twinning project for viral haemorrhagic septicaemia (VHS) with Korea and Denmark. The 2nd International Workshop for Research of VHS and Rhabdoviral diseases.

12) **H. J. Kim**, J. J. Han, T. Taksdal, O. B. Dale, **N. J. Olesen**, J. S. Park, S. R. Kwon. 2017. Severe mortality of farmed rainbow trout caused by infectious haematopoietic

necrosis virus in Korea. The 2nd International Workshop for Research of VHS and Rhabdoviral diseases.

13) **H. J. Kim**, **N. J. Olesen**. 2017. Assessment of a new conventional RT-PCR for VHSV detection in an inter-laboratory proficiency test. The 2nd International Workshop for Research of VHS and Rhabdoviral diseases, Korea.

14) S. R. Kwon, J. S. Park, **H. J. Kim**. 2017. Transcriptome analysis of olive flounder, *Paralichthys olivaceus* immunized with Poly(I:C)-VHSV or formalin-killed VHSV. The 2nd International Workshop for Research of VHS and Rhabdoviral diseases, Korea.

15) **N. J. Olesen**, A. L. F. Alencar, A. Cuenca, **H. J. Kim**, T. Ito. 2017. Viral haemorrhagic septicaemia virus (VHSV): On the search for determinants important for virulence in rainbow trout *Oncorhynchus mykiss*. The 2nd International Workshop for Research of VHS and Rhabdoviral diseases, Korea.

16) **H. J. Kim**, S. S. Mikkelsen, **N. J. Olesen**. 2016. Development of a novel one-step reverse transcription PCR method for detecting viral haemorrhagic septicaemia virus. 20th Annual workshop of the EU National Reference Laboratories for Fish Diseases, Denmark.

17) **H. J. Kim**, S. S. Mikkelsen, **N. J. Olesen**. 2016. Development of a novel one-step RT-PCR for detection of viral haemorrhagic septicaemia virus. The 1st International Workshop on Viral Hemorrhagic Septicemia Research in Korea; 2016 April 21; Busan, Korea.

18) **H. J. Kim**, **N. J. Olesen**. 2016. The status of the OIE Laboratory Twinning project for viral haemorrhagic septicaemia (VHS) with Korea and Denmark. The 1st International Workshop on Viral Hemorrhagic Septicemia Research in Korea; 2016 April 21; Busan, Korea.

- **Poster Presentations**

1) **H. J. Kim**, S. R. Kwon. 2016. Constructions of chimeric PCR positive plasmids for conventional PCR detection of all OIE listed aquatic animal pathogens. Korean Federation of Fisheries Sciences and Technology Societies (KOFFST) International Conference in Korea.

2) **H. J. Kim**, J. S. Park, J. H. Kim, J. Y. Kim, H. D. Song, S. W. Chon, S. R. Kwon. 2016. Effect of formalin on the infectivity of viral hemorrhagic septicaemia virus (VHSV). Symposium on Journal of Fish Pathology in Korea.

3) **H. J. Kim**, J. H. Kim, J. S. Park, H. L. Lee, S. R. Kwon. 2015. Effect of fetal bovine serum (FBS) concentration on the infectivity of viral hemorrhagic septicemia virus (VHSV). KOFFST International Conference in Korea.

4) **H. J. Kim**, K. J. Ahn, J. S. Park, H. L. Lee, S. R. Kwon. 2015. Seaweed mix powder (MEGA SMART KELP) exhibited *in vitro* antiviral activity against viral hemorrhagic septicemia virus but not against infectious pancreatic necrosis virus. International Symposium on World Aquaculture in Korea.

5) **H. J. Kim**. 2015. 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. 17th International Conference on Diseases of Fish and Shellfish; 2015 September 7-11; Las Palmas, Spain.

4.2. Training and consultation experience for the disease during OIE Twinning project

4.2.1. Kick-off meeting for OIE Twinning project at DTU in Denmark (July 2015)

4.2.2. Meeting of the OIE Twinning project participants at Bulletin of the European Association of Fish Pathologists (EAFP Conference, Gran Canaria, Spain) (September 2015)

4.2.3. Research cooperation for development of the novel VHSV detection method using conventional RT-PCR method at DTU (December 2015–February 2016)

4.2.4. The 2nd research cooperation for development of the novel VHSV detection method using conventional RT-PCR method at DTU (March 2016)

4.2.5. Meeting of the OIE Twinning project participants for preparing the project schedule in 2017 (October 2016)

4.2.6. Meeting of the OIE Twinning project participants with general directors between Korea and Denmark (March 2017)

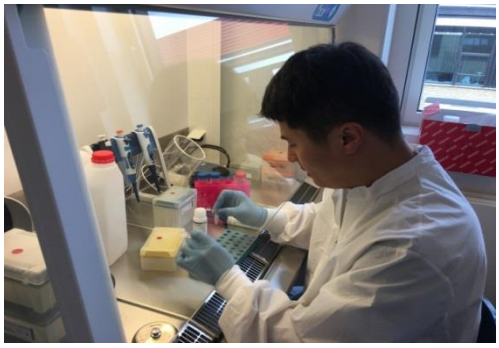
4.2.7. Meeting of the OIE Twining project participants for preparing the project schedule (October 2017)

Meeting to prepare the program schedules for OIE Twinning project

Meeting between the directors on both sides

4.2.8. Research cooperation for developing the novel VHSV detection and genotyping method using real time RT-PCR at DTU, Denmark (September 2017–December 2017)

CPE observation based on VHSV titration



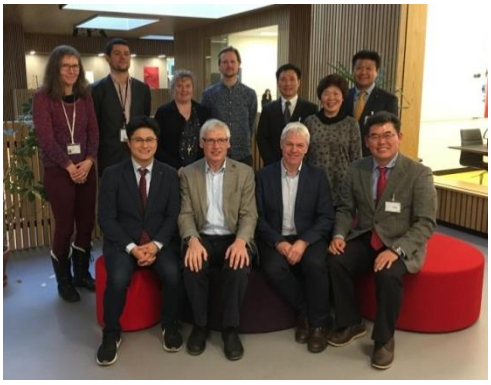
RNA extraction for development of the novel VHSV detection and genotyping method

4.2.9. Pathogenicity trials of two Korean IHNV isolates (phylogenetically different) using rainbow trout at DTU, Denmark (September 2017–December 2017)

Pathogenicity check of Korean IHNV by Dr. Kim

Infected rainbow trout with IHNV

4.2.10. Final meeting and workshop of OIE Twinning project between Denmark and Korea (11 December 2017)



Participants in final meeting of OIE Twinning project

Speech of thanks from General Director of NFQS to NVI

Introduction about application of OIE Reference Laboratory of NFQS by Prof. Olesen

Presentation about outcome of OIE Twinning project by Dr. KIM

4.2.11. Memorandum of Agreement (MOA) between NVI of Denmark and NFQS of Korea (11 December 2017)

Signed by both director general in MOA draft

Group photo after ceremony for MOA

4.3. Trainings, lectures and consultations

4.3.1. First training session of Ecuador inspectors on diagnostic methods through the ODA program at NFQS (September 2015)

4.3.2. Second training session of Ecuador inspectors on diagnostic methods through the ODA program at NFQS (August 2016)

4.3.3. Training on OIE Reference Laboratory (salmon alphavirus) and collaboration center (risk accessment) and development of joint research tasks at the National Veterinary Institute in Norway (April 2016)

4.3.4. Training on the operation system, problems, and know-hows of the OIE Reference Laboratory in Japan (February 2017)

4.3.5. Presentation about the OIE Twinning Project and chimeric plasmid PCR positive control in Japan (January 2017)

4.3.6. Consultations for activation of OIE activities with Dr. Kugita at the OIE Regional Representation for Asia and the Pacific and consultations on the novel VHSV detection method with Dr. Crane and Dr. Moody at the conference of OIE Reference Laboratory in Tokyo (February 2017)

4.3.7. Consultations on the OIE Twinning Project with the chairman and a member of the OIE Aquatic Animal Commission (May 2017)

4.3.8. Lecture on the OIE and OIE Twinning Project to 16 ODA recipient countries of KOICA (March 2017)

4.3.9. Participation of the diagnostic training course at EU Reference Laboratory of fish diseases (October 2017)

Meeting with other participants

Participation in the diagnostic training course

4.4. List of scientific meetings

4.4.1. Scientific meeting for diagnostic method using conventional PCR and cell culture in Japan (April 2014)

4.4.2. Participation at OIE General Assembly (May 2014, 2015, 2016, 2017)

4.4.3. Scientific meeting at the 9th International Symposium on Viruses of Lower Vertebrates (ISVLV, October 2014)

4.4.4. Host of the invitation event with 15 experts from OIE Reference Laboratories in NFQS (October 2014)

4.4.5. Invited meeting with Prof. N. J. Olesen (VHS) at NFQS (October 2014)

4.4.6. Invited meeting with Dr. Yuasa (KHV) and Dr. Kawato (RSIV) at NFQS (October 2014)

4.4.7. Participation at the 3rd OIE Global Conference on Aquatic Animal Health (January 2015)

4.4.8. Participation in workshop on aquatic focal point (January 2015)

4.4.9. Invited meeting with German experts on fish pathology at NFQS (September 2015)

4.4.10. Discussion on the NFQS analysis system and facility through EU TAIEX program (November 2015)

4.4.11. The 1st International Workshop on Viral Haemorrhagic Septicaemia Research in Korea (April 2016)

4.4.12. Oral presentation about the novel VHSV conventional RT-PCR method at the EURL workshop (June 2016)

4.4.13. Scientific discussion at OIE conference between Reference Laboratory experts in Tokyo (February 2017)

4.4.14. Experimental discussion with Ministry of New Zealand for Primary Industries at AAQ laboratory in NFQS (April 2017)

4.4.15. Scientific meeting of VHS experts and tour of the AAQ laboratory at NFQS (May 2017)

4.4.16. The 2nd International Workshop on Viral Haemorrhagic Septicaemia Research in Korea (May 2017)

4.4.17. Participation in the OIE General Assembly and Aquatic Commission pre-meeting (May 2017)

4.4.18. Scientific meeting of teams for OIE Twinning projects between China (IHN with USA) and Korea (VHS with Denmark) (May 2017)

4.4.19. Scientific meeting and oral presentation at the 21st Annual Workshop of the EU Reference Laboratory (May 2017)

4.4.20. Scientific meeting and oral presentation at the 10th International Symposium on Viruses of Lower Vertebrates (ISVLV, June 2017)

4.4.21. Oral presentation on the OIE Twining Project between Japan and Indonesia for koi herpesvirus (August 2017)

Presentation about the status of the OIE Twining Project between Korea and Denmark

Group photo of all the participants

4.4.22. Scientific meeting and oral presentation at the 18th International Conference on Diseases of Fish and Shellfish (September 2017)

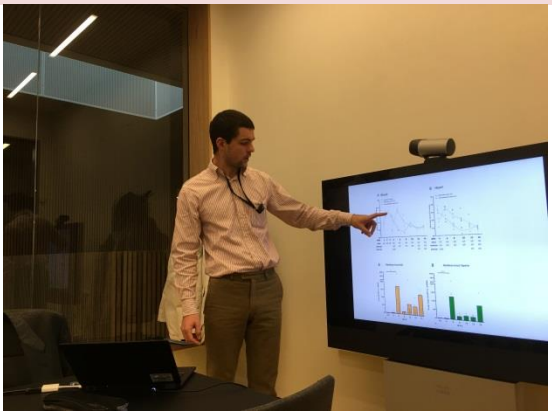
Meeting with other participants

Presentation about validation of the novel VHSV gene detection method based on conventional RT-PCR

4.4.23. Scientific meeting for OIE Twinning project and further researches at DTU (December 2017)

Discussion about further cooperation research between NVI and NFQS

Discussion about detail plans of research in 2018



Presentation about current PRV research by Dr. Vendramin

Presentation about research of red mark syndrome by Dr. Schmidt

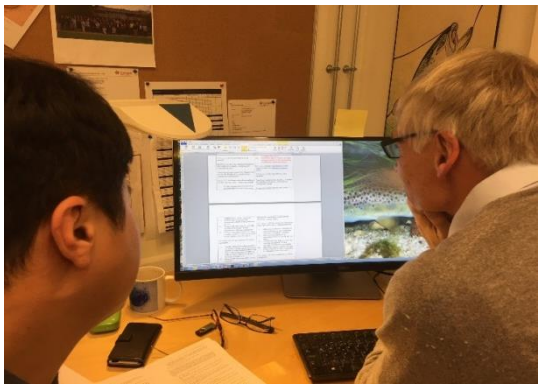
4.4.24. Scientific meeting about control of aquatic animal diseases in India at DTU (December 2017)

Presentation by Mr. Dhamotaran (Ph.D.student) from Oslo

Group photo with Mr. Dhamotaran and DTU members

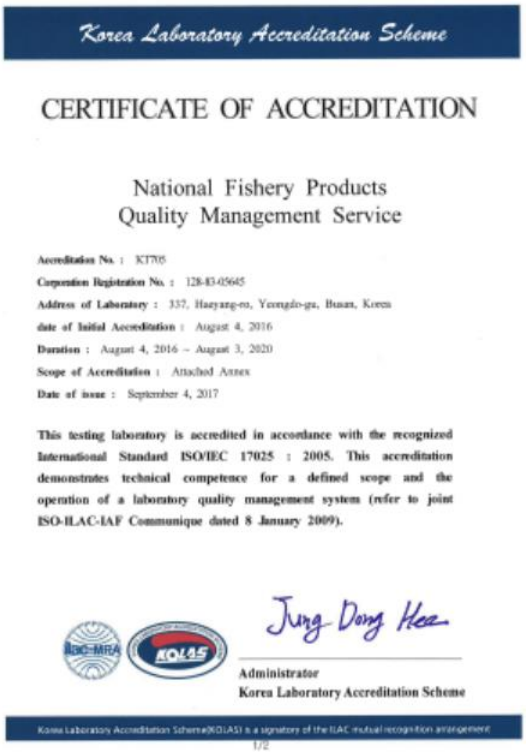
4.4.25. Meeting for amendments in the Chapter 2.3.10 on Viral Haemorrhagic Septicaemia (VHS) in the OIE Aquatic Manual (December 2017)

Meeting between Prof. Olesen and Dr. Kim at DTU



Discussion about the OIE aquatic manual including conventional RT-PCR method (3F2R)

4.5. Certificates of accreditation to the ISO 17025 (August 2016)



Korea Laboratory Accreditation Scheme

CERTIFICATE OF ACCREDITATION

National Fishery Products
Quality Management Service

Accreditation No. : KT705
Corporation Registration No. : 128-83-05645
Address of Laboratory : 337, Haeyang-ro, Yeongdo-gu, Busan, Korea
date of Initial Accreditation : August 4, 2016
Duration : August 4, 2016 ~ August 3, 2020
Scope of Accreditation : Attached Annex
Date of issue : September 4, 2017

This testing laboratory is accredited in accordance with the recognized International Standard ISO/IEC 17025 : 2005. This accreditation demonstrates technical competence for a defined scope and the operation of a laboratory quality management system (refer to joint ISO-ILAC-IAF Communique dated 8 January 2009).

Jung Dong Hee

Administrator
Korea Laboratory Accreditation Scheme

Korea Laboratory Accreditation Scheme(KOLAS) is a signatory of the ILAC mutual recognition arrangement

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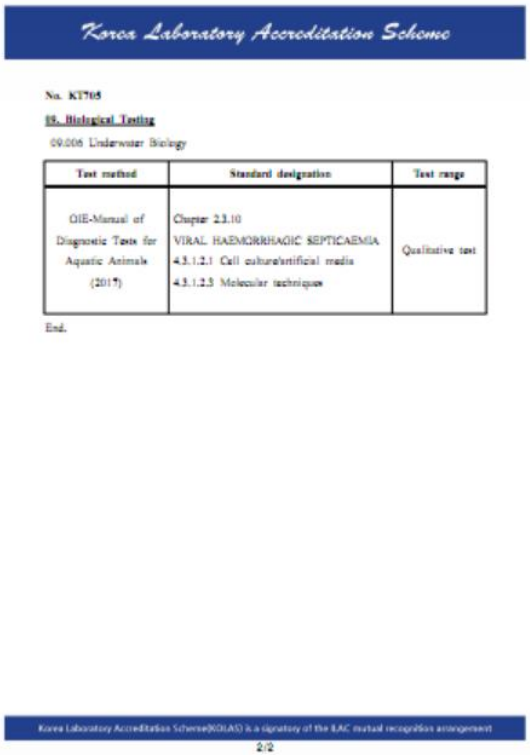

Korea Laboratory Accreditation Scheme

No. KT705

15. Biological Testing

09.006 Underwater Biology

Test method	Standard designation	Test range
OIE-Manual of Diagnostic Tests for Aquatic Animals (2017)	Chapter 2.3.10 VIRAL HAEMORRHAGIC SEPTICAEMIA 4.3.1.2.1 Cell culture/artificial media 4.3.1.2.3 Molecular techniques	Qualitative test

End.

Korea Laboratory Accreditation Scheme(KOLAS) is a signatory of the ILAC mutual recognition arrangement

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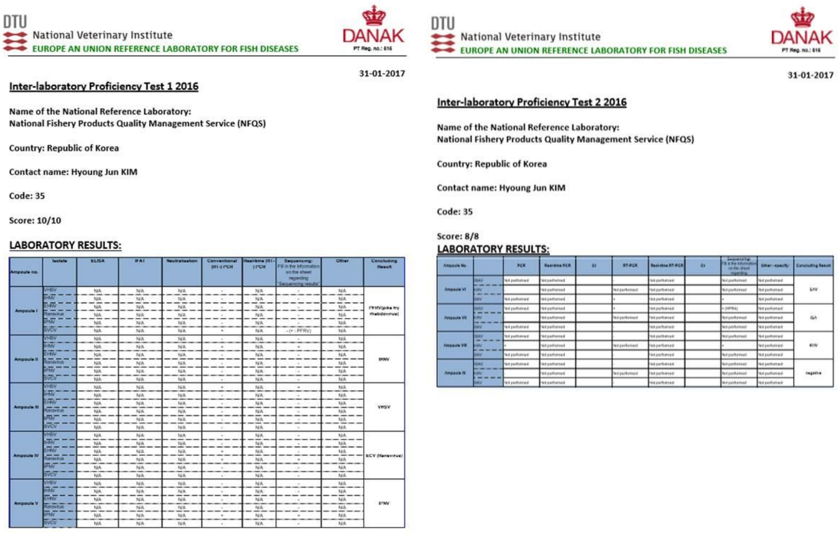
Certificate of accreditation for VHS diagnostic methods and system by ISO/IEC 17025

4.6. EURL proficiency test from 2014 to 2017 by EU Reference Laboratory (DTU, Denmark)


DTU
National Veterinary Institute
EUROPE AN UNION REFERENCE LABORATORY FOR FISH DISEASES
DANAK
PT Reg. no.: 616

31-01-2017

Inter-laboratory Proficiency Test 1 2016

Name of the National Reference Laboratory:
National Fishery Products Quality Management Service (NFQS)

Country: Republic of Korea

Contact name: Hyoung Jun KIM

Code: 35

Score: 10/10

LABORATORY RESULTS:

Ampoule no.	Isolate	ELISA	IFAT	Neutralisation	Conventional (RT-) PCR	Real-time (RT-) PCR	Sequencing: Fill in the information on the sheet regarding "Sequencing results"	Other	Concluding Result
Ampoule I	VHSV	N/A	N/A	N/A	-	N/A	-	N/A	PFRV(pike fry rhabdovirus)
	IHNV	N/A	N/A	N/A	-	N/A	-	N/A	
	EHNV	N/A	N/A	N/A	-	N/A	-	N/A	
	Ranavirus	N/A	N/A	N/A	-	N/A	-	N/A	
	IPNV	N/A	N/A	N/A	-	N/A	-	N/A	
	SVCV	N/A	N/A	N/A	+	N/A	- (+ : PFRV)	N/A	
Ampoule II	VHSV	N/A	N/A	N/A	-	N/A	-	N/A	IHNV
	IHNV	N/A	N/A	N/A	+	N/A	+	N/A	
	EHNV	N/A	N/A	N/A	-	N/A	-	N/A	
	Ranavirus	N/A	N/A	N/A	-	N/A	-	N/A	
	IPNV	N/A	N/A	N/A	-	N/A	-	N/A	
	SVCV	N/A	N/A	N/A	-	N/A	-	N/A	
Ampoule III	VHSV	N/A	N/A	N/A	+	N/A	+	N/A	VHSV
	IHNV	N/A	N/A	N/A	-	N/A	-	N/A	
	EHNV	N/A	N/A	N/A	-	N/A	-	N/A	
	Ranavirus	N/A	N/A	N/A	-	N/A	-	N/A	
	IPNV	N/A	N/A	N/A	-	N/A	-	N/A	
	SVCV	N/A	N/A	N/A	-	N/A	-	N/A	
Ampoule IV	VHSV	N/A	N/A	N/A	-	N/A	-	N/A	ECV (Ranavirus)
	IHNV	N/A	N/A	N/A	-	N/A	-	N/A	
	EHNV	N/A	N/A	N/A	+	N/A	-	N/A	
	Ranavirus	N/A	N/A	N/A	+	N/A	+	N/A	
	IPNV	N/A	N/A	N/A	-	N/A	-	N/A	
	SVCV	N/A	N/A	N/A	-	N/A	-	N/A	
Ampoule V	VHSV	N/A	N/A	N/A	-	N/A	-	N/A	IPNV
	IHNV	N/A	N/A	N/A	-	N/A	-	N/A	
	EHNV	N/A	N/A	N/A	-	N/A	-	N/A	
	Ranavirus	N/A	N/A	N/A	-	N/A	-	N/A	
	IPNV	N/A	N/A	N/A	+	N/A	+	N/A	
	SVCV	N/A	N/A	N/A	-	N/A	-	N/A	

DTU
National Veterinary Institute
EUROPE AN UNION REFERENCE LABORATORY FOR FISH DISEASES
DANAK
PT Reg. no.: 616

31-01-2017

Inter-laboratory Proficiency Test 2 2016

Name of the National Reference Laboratory:
National Fishery Products Quality Management Service (NFQS)

Country: Republic of Korea

Contact name: Hyoung Jun KIM

Code: 35

Score: 8/8

LABORATORY RESULTS:

Ampoule No.		PCR	Real-time PCR	Ct	RT-PCR	Real-time RT-PCR	Ct	Sequencing: Fill in the information on the sheet regarding	Other - specify	Concluding Result
Ampoule VI	ISAV	Not performed	Not performed		-	Not performed		Not performed	Not performed	SAV
	KHV		Not performed		Not performed	Not performed		Not performed	Not performed	
	SAV	Not performed	Not performed		+	Not performed		+	Not performed	
Ampoule VII	ISAV	Not performed	Not performed		+	Not performed		+ (HPR4)	Not performed	ISA
	KHV		Not performed		Not performed	Not performed		Not performed	Not performed	
	SAV	Not performed	Not performed		-	Not performed		Not performed	Not performed	
Ampoule VIII	ISAV	Not performed	Not performed			Not performed		Not performed	Not performed	KHV
	KHV	+	Not performed		Not performed	Not performed		+	Not performed	
	SAV	Not performed	Not performed		-	Not performed		Not performed	Not performed	
Ampoule IX	ISAV	Not performed	Not performed			Not performed		Not performed	Not performed	negative
	KHV		Not performed		Not performed	Not performed		Not performed	Not performed	
	SAV	Not performed	Not performed		-	Not performed		Not performed	Not performed	

Feedback on the EURL Proficiency Test Scheme 2016 from DTU, Denmark

4.7. Validation and Standardization of diagnostic methods for VHS

Dr. Kim validated the conventional reverse-transcription (RT-PCR) method on VHSV I and IVa genotypes using one- and two-step conventional RT-PCR methods listed in the OIE manual of diagnostic tests.

According to this OIE Twinning project, Dr. Kim and Prof. Olesen validated a novel conventional RT-PCR method (3F2R) for detection of all VHSV genotypes and suggested that the new method replace the current VN primer set recommended for VHSV detection by the OIE manual of diagnostic tests for aquatic animals.

In addition, the NFQS has optically validated the serological methods for VHSV confirmation using several commercial antibodies during OIE Twinning project.

4.8. Possession of all VHSV genotypes in the AAQ laboratory

The NFQS possesses all genotypes of live 25 VHSV isolates that were received from the VHSV OIE Reference Laboratory in Denmark. NFQS can permanently make the VHSV

fluid by implementing the cell culture method and thus prepare the positive control virus. This virus will be provided upon request from any country and laboratory.

Table. List of received VHSV genotypes by the AAQ lab from the OIE Reference Laboratory for VHS

Num.	Genotype	Isolate name	Country	Year	Fish Fresh, Bracksh or Sea	Reference or Source
1	I	DK-F1	Denmark	1962	Rainbow trout (F)	Jensen (1965)
2	I	DK-297 (Hededam)	Denmark	1970	Rainbow trout (F)	Vestergård Jorgensen (1974)
3	Ia	AU-8/95	Australia			University of Veterinary Medicine, Vienna (unpubl.)
4	Ia	PL-202473	Poland			National Veterinary Research Institute, Pulawy, Poland (unpubl.)
5	Ib	DK-1p8	Denmark	1996	Herring (S)	Mortensen et al. (1999)
6	Ib	DK-4p37	Denmark			Mortensen et al. (1999)
7	Ib	SE-SVA-1033	Sweden	2000	Rainbow trout (S)	Nordblom & Norell (2000)
8	Ic	DK-5131	Denmark			Jonstrup et al. (2009)
9	Ic	DK-5123	Denmark			Jonstrup et al. (2009)
10	Id	FIN-2ka66/2000	Finland			Einer-Jensen et al. (2004)
11	Id	NO-A163-68EG46	Norway			Håstein, Holt & Krogsrud (1968)
12	Ie	GE-1.2	Russia			Lab of Aq. Animal Health, Russia Res. Institute(unpubl.)
13	Ie	TR206239-1	Turkey			Ito et al. (2012)
14	II	DK-1p52	Denmark	1996	Sprat (S)	Mortensen et al. (1999)
15	II	DK-1p53	Denmark			Mortensen et al. (1999)
16	III	DK-4p168	Denmark	1996	Atlantic herring (S)	Mortensen et al. (1999)
17	IIIa	DK-4p51	Denmark			Mortensen et al. (1999)
18	IIIa	FR-L59x	France	1987	Eel (F)	Thiery et al. (2002)
19	IIIb	NO-2007-50-385	Norway			Dale et al. (2009)
20	IVa	JF-JF00Ehil	Japan	2000		Nishizawa et al. (2002)
21	IVa	CAN-99-019	Canada	1999	Sardine (S)	Ito et al. (2012)
22	IVa	KJ2008	Korea			Kim and Kim (2011)
23	IVb	Goby 1-5	USA	2006	Round goby (F)	Groocock et al. (2007)
24	IVb	Blue gill, Budd Lake, MI	USA	2007	Bluegill (F)	USGS (unpubl.)
25	IVc	New Brunswick	USA	2000	Mummichog (F)	Groocock et al. (2007)

4.9. Design of the sample preparation method for molecular biological detection by PT test

For the VHSV proficiency test (PT) using conventional RT-PCR (3F2R method), the VHSV samples were prepared by Dr. Kim and Prof. Olesen using FTA cards (Fig. 1). The reproducibility and robustness of the 3F2R method test was validated by nine selected laboratories, CEFAS (UK), ANSES (France), IZSVe (Italy), FLI (Germany) at two laboratories, DTU (Denmark), NRIA (Japan) at two laboratories, and NFQS (Korea). As FTA cards inactivate the pathogen while preserving the nucleic acid intact, FTA cards are more suitable for sending pathogen samples without import permit.

Fig. 1. Preparation of PT samples for VHSV gene detection using FTA cards.

4.10. Production of chimeric PCR positive control and VHSV detection kit using real-time PCR of PNA probe and constructed chimeric plasmid

To prevent misdiagnosis from positive-control gene contamination, chimeric PCR plasmids of all OIE-listed diseases, including VHS, were constructed by Dr. Kim for the conventional PCR method described in the OIE diagnostic manual. This technique for production of chimeric PCR plasmids by the NFQS was patented and transferred to SEASUN Bio Corporation. Thus, this company produces chimeric PCR positive plasmids of all listed diseases (Fig. 2a) and the VHSV detection kit using real-time PCR of PNA probe using Dr. Kim's patents (Fig. 2b).

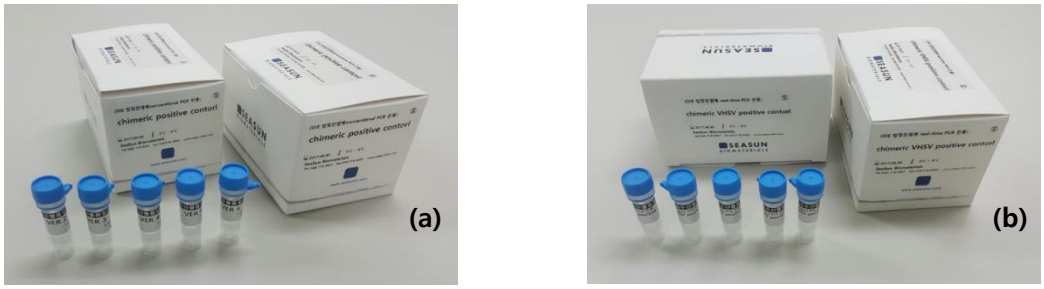


Fig. 2. Products of chimeric PCR positive control (Fig. 2a) and VHSV detection kit using real time PCR of PNA probe (Fig. 2b).

5. Situation in Candidate Centre at the end of the project including the ability to maintain and sustain the achieved objectives

The NFQS earned international accreditation of ISO/IEC 17025 on diagnostic method of VHS during the project. And Dr. Kim of the NFQS published a book on fish diseases and six research papers on VHS in the peer-review journals, and delivered oral presentations with 18 different themes and poster presentations with five different themes at international workshops and symposiums. These processes have helped him to build a network with specialists internationally in the field of aquatic animals' disease. In fact, he already forged a relationship with OIE-associated experts even before the start of this project by inviting 14 experts from OIE Reference Laboratory for Aquatic Animals to the 2014 Global Conference of OIE Reference Laboratories and Collaborating Centers, held in Song-do of Incheon in Korea. He also led multiple educational programs that contributed to strengthening diagnostic methods of aquatic animal diseases in other countries, and performed the international proficiency test from the EURL (European Union Reference Laboratory for fish, parent laboratory) four times for four years, showing a stellar performance.

Dr. Kim scientifically proved that the conventional RT-PCR presented in the OIE manual of diagnostic tests for VHS is unsuitable for detecting VHSV (genotype IVa) in Asia, and published the results of it in the international journal (Aquaculture, 2015). And his team collaborated with the team of parent laboratory to develop a novel conventional RT-PCR (3F2R method), which fixed several problems of the current conventional RT-PCR for VHS, through standardization and validation of diagnostic method—the most important goal for this OIE Twinning project. The best accomplishment from the project is that Dr. Kim and his team of the NFQS could learn know-hows of the standardization and validation of parent laboratory.

Dr. Kim and Prof. Olesen submitted a proposal to the OIE in Dec. 2017 to amend the present OIE manual of diagnostic tests for VHS based on the 3F2R method.

Dr. Kim has accumulated a variety of experience—doing research on VHS, giving multiple presentations at global conferences to become an international specialist, having international exchange, conducting inspection according to ISO/IEC 17025 to earn credibility of diagnostic results, and developing and evaluating diagnostic methods. Based on the experience and results from them, he submitted an application for the designation as OIE Reference Laboratory for VHS Dec. 28th of 2017 and was endorsed by the OIE Aquatic Animal Health Standards Commission and OIE council.

6. Lessons learned to improve future projects

The NFQS, a candidate laboratory aiming for the designation as OIE Reference Laboratory through this OIE Twinning project, revealed the current situation its lab is facing to OIE Reference Laboratory, a parent laboratory for the NFQS. It enabled the parent laboratory to evaluate what the candidate laboratory really lacks and needs to improve to become a OIE Reference Laboratory for VHS. An important lesson here is that when a parent laboratory visits a candidate laboratory, they need to be able to see what the candidate really goes through; the real situation is, etc.

The NFQS could set the plan for OIE Twinning project thanks in great part to the evaluation. In fact, the candidate laboratory made impractical plans to just increase the number of paper published regarding VHS before the project began, but changed them based on mutual communication and comments from OIE Aquatic Animal Health Standards Commission. What we learned from the experience is that it is important to draw a big picture for research and make a proper evaluation, rather than simply focusing on raising the number of research or experiment conducted.

Meanwhile, experts from both institutes realized that the continued collaborative research even at the end of the project encouraged them to achieve more.

Therefore, the best part of OIE Twinning project between Korea and Denmark was that we share the same goal of controlling diseases and could fight against the problem as one despite the long distance and different culture.

7. Recommendations for future projects

We could understand why OIE created OIE Twinning project as an official project—different countries have different characteristics of pathogenic agents and genotypes, and it needs researchers from different parts of the world to gather together to conduct collaborative study and share information for mutual growth.

Indeed, the conventional RT-PCR now presented in OIE diagnostic manual is suitable for diagnosing VHSV genotype in Europe, whereas unsuitable for diagnosing VHSV genotype IVa in Asia as they showed a very low sensitivity of viral gene detection. We developed and validated a new method (3F2R) to fix the problem, which was scientifically proven to have the same detection sensitivity as real-time PCR and cell culture methods. Based on the results, we submitted an amendment for OIE manual of diagnostic tests to OIE Aquatic Animal Health Standards Commission in Dec. 2017.

Considering that Korea has not managed VHS quite well so far, we are very much grateful for OIE to create this project and let the parent laboratory give us a lot of advice and educational program. It was so helpful for the NFQS of Korea to strengthen its capability.

From the candidate laboratory perspective, however, we underwent some problems. OIE Twinning project covers not only the involved institutes but also two countries from different regions, and is known all around the world by the announcement of OIE. But during the project, there was a change in person in charge—a head of the aquatic animal quarantine division—while the NFQS continued its work with aims to be designated as OIE Reference Laboratory. Besides, the person in charge expressed intentions to designate a different laboratory, as opposed to us NFQS, as OIE Reference Laboratory on the same disease (VHS). Worse yet, his influence put pressure on a Korean CVO to make changes in choosing a laboratory for the designation.

To be sure, there is no regulation regarding the OIE Reference Laboratory designation saying a laboratory working in OIE Twinning project certainly becomes OIE Reference Laboratory, and different laboratories in one country are not allowed to apply for the designation. However, if it is okay for another laboratory to apply for the designation as OIE Reference Laboratory on the same disease while the already-applying laboratory (us NFQS) submitted the application to OIE and was known to the world to be designated, it seems nobody would try for OIE Twinning project.

The candidate laboratory, namely NFQS, tried to figure things out at the time with clear answers from OIE for this matter, but what we heard back from OIE was that it is the job of the country themselves to deal with the application for OIE Reference Laboratory, which in turn put even bigger pressure on a CVO of our country. In this regard, we are wondering what the real goal or meaning is for OIE Twinning project. We do not think OIE Twinning project is a country's matter to deal with. Rather, we believe that it is the project two different countries cooperate to reach the same goal, meaning any unnecessary pressure should not be followed.

We strongly believe that OIE needs to have a clear regulation that prevents any changes or problems from derailing the already-set project team, so that OIE Twinning project could

further develop going forward. In this context, we would like to suggest that OIE draws up clear guidelines that could protect candidate laboratory, which aim for being designated as OIE Reference Laboratory, from going through any difficulties or pressure from outside.

8. Mid to long term strategy for the Candidate Centre to maintain benefits, capacity, and a sustainable link with the Parent laboratory.

Through OIE Twinning project this time, both institutes could make a close relationship much stronger. As VHS have different characteristics from different genotypes and regions, both institutes concluded a MOA on Dec. 11th of 2017 to keep collaborating in research on fish viral disease including VHS. Therefore, both institutes will continue to communicate in various ways including holding a regular workshop and experts exchange programs by making plans of them annually. Two heads of the institutes will get together at the end of every year to evaluate the results. Ensuring the budget for these jobs is also planned.