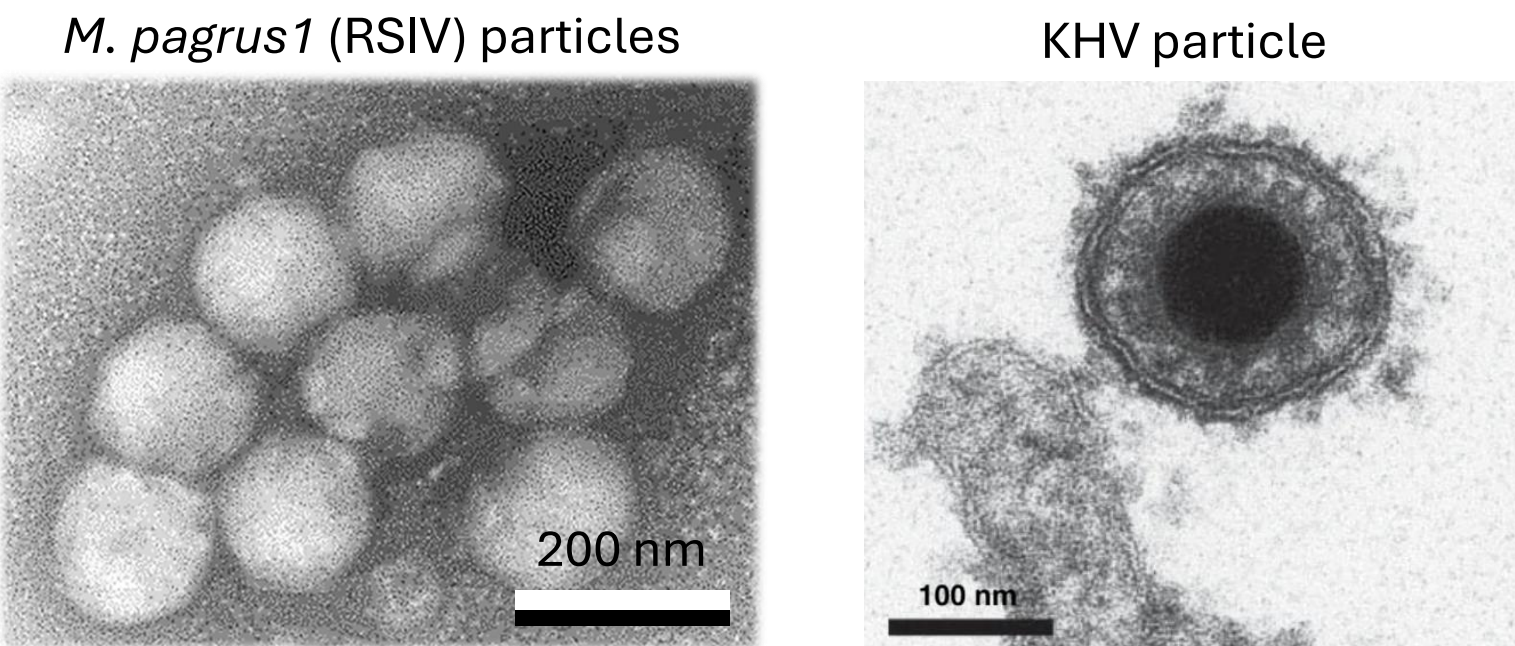


WOAH Reference Laboratory for Infection with *Megalocytivirus pagrus1* and koi herpesvirus

[Fisheries Technology Institute (FTI), Japan Fisheries Research and Education Agency (FRA)]

1. Overview

Infection with *Megalocytivirus pagrus1* (formerly red sea bream iridovirus) is a major concern to the global aquaculture industry due to its wide host range (> 50 fish species) and potential spread through international live fish trade, including ornamental fish. Infection with koi herpesvirus (KHV), is a causative agent of mass mortality events in ornamental fancy carp (koi) and common carp. It is a major threat in koi and carp industries worldwide.



2. Expertise, services and facilities (photos)

Our laboratory is engaged in the diagnosis, surveillance, and research of important viral and bacterial diseases affecting aquatic animals, including *M. pagrus1* and KHV as the WOAHP RL.

- ✓ **Expertise:** diagnosis, virology (isolation, titration, etc.), epidemiology, biosecurity management
- ✓ **Facilities:** PCR clean bench, biosafety cabinet for cell culture and virus handling, and a wet laboratory for infection challenge experiments.
- ✓ **Services:** confirmatory diagnosis, positive materials (DNA, virus isolate, antibody, cell line), technical training (on site), providing disease information (remote)

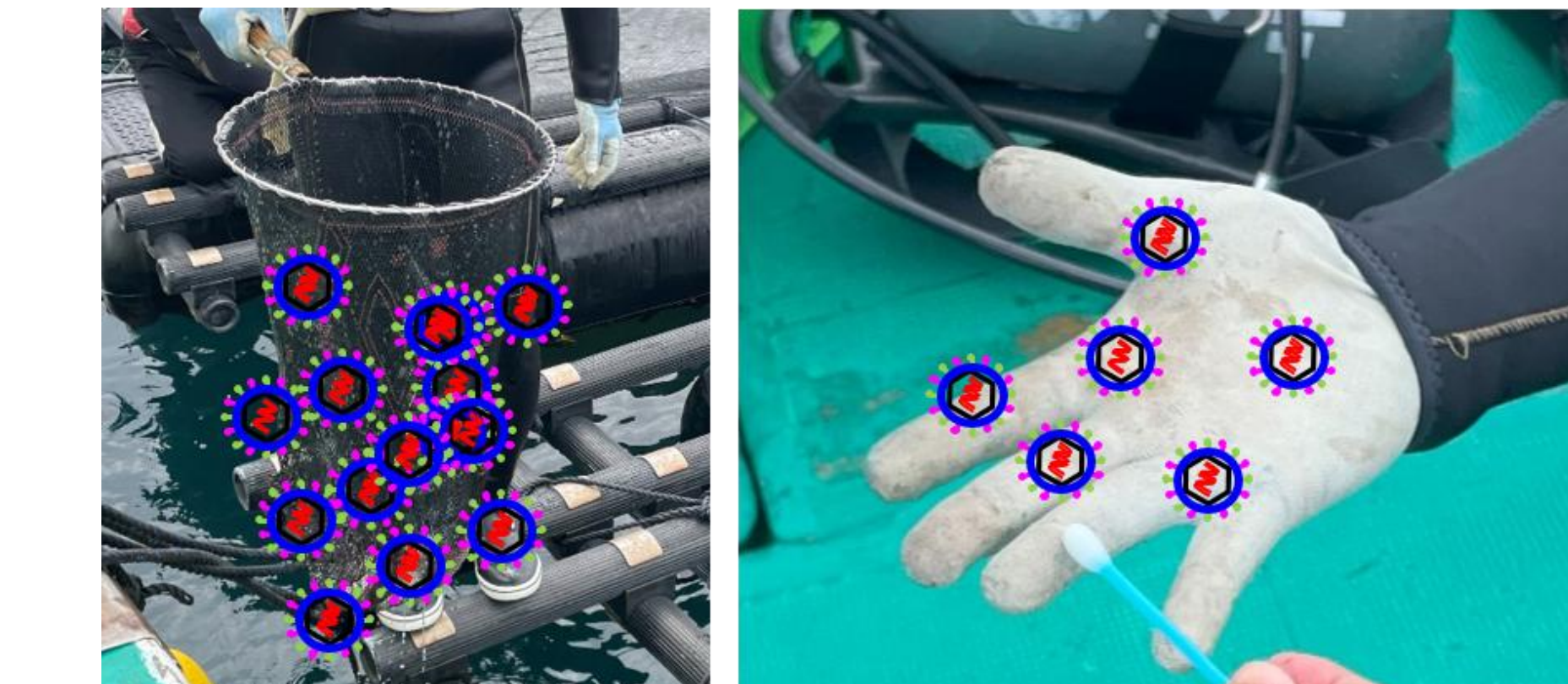
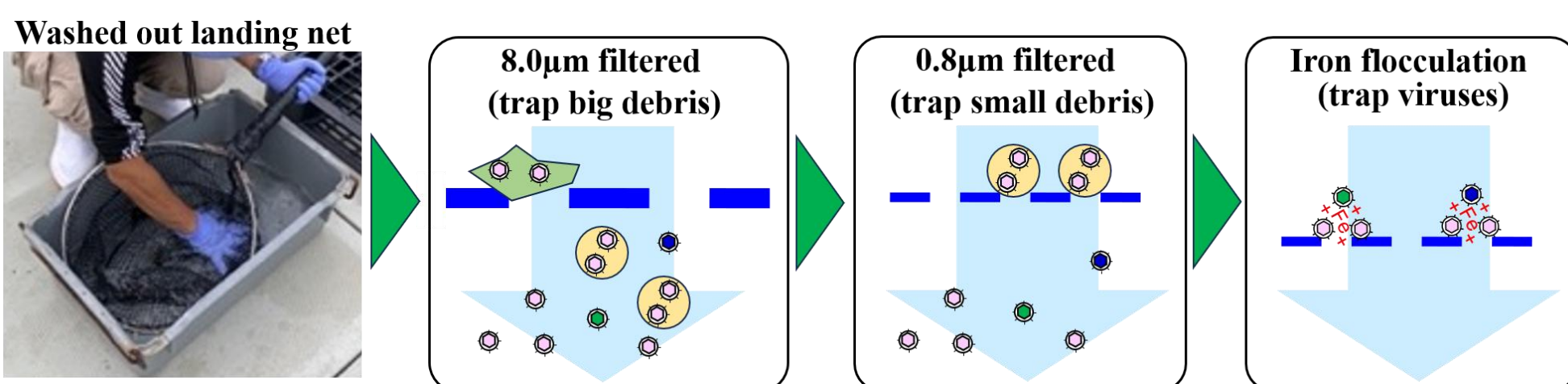
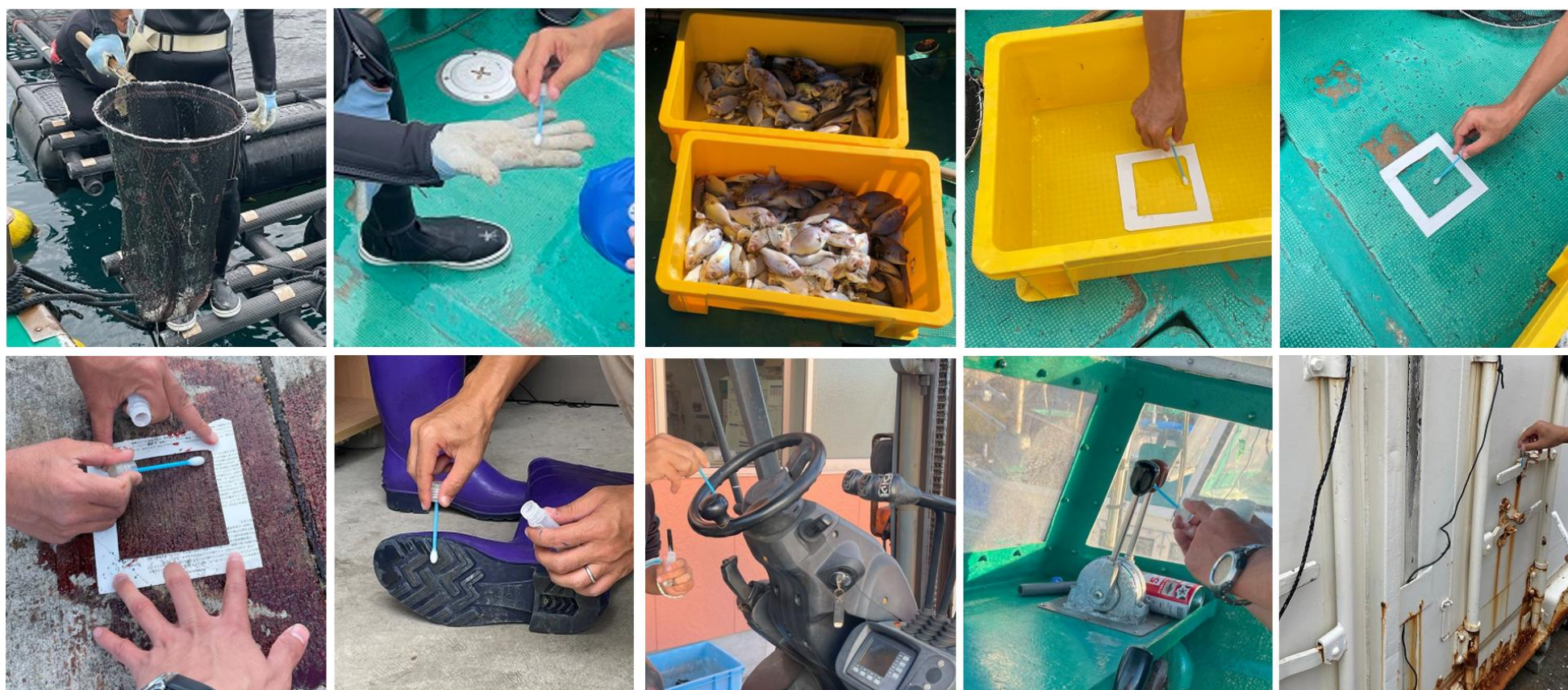


3. Recent Innovations, research highlights, technical developments

Fomite transmission of *M. pagrus1*

Fomite transmission could be an important risk factor for spreading *M. pagrus1* infection between farming units rather than environmental water transmission.

Kawato *et al.* (2025) *J Fish Dis* 48:e14103



Fish landing net and gloves which are used for collecting dead fish (carcasses) were highly contaminated with *M. pagrus1* (RSIV).

Inactivation of *M. pagrus1*

Inactivation characteristics of *M. pagrus1* (RSIV) was evaluated under various physical conditions and disinfectant treatments to inform the implementation of appropriate hygiene management practices on fish farms.

Kawato *et al.* (2026) *Fish Pathol* 61:1-10

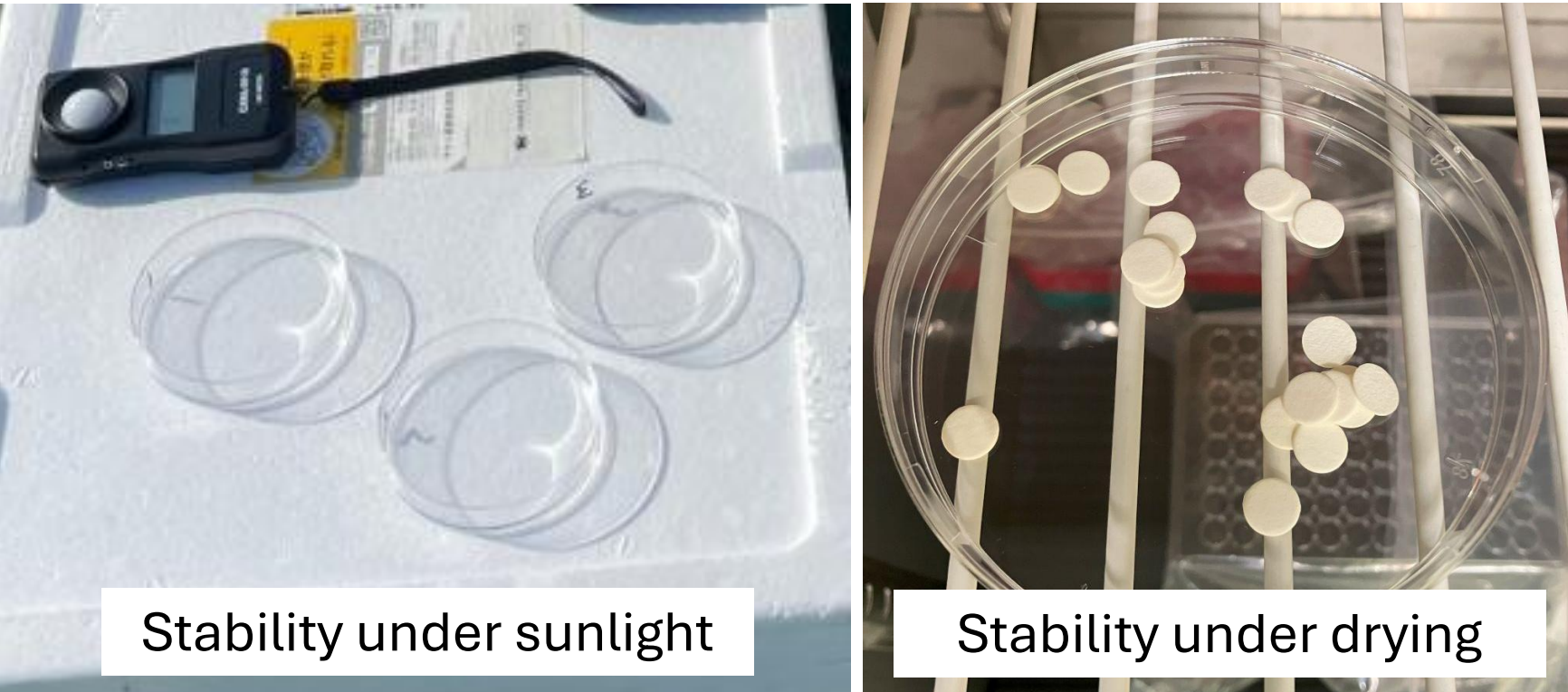


Table. Summary of inactivation conditions of *M. pagrus1* (RSIV)

Treatment	Inactivation conditions*1
Natural seawater	30°C for 2 days; 25 and 20°C for 7 days
Sunlight	>1 h
Drying	>60 days
Heat	50°C for 30 min or 60°C for 30 s
Ethanol	40% for 30 s
Electrolyzed seawater*2	0.14 ppm for 60 min
Sodium hypochlorite (1)*2	0.5 ppm for 30 s or 0.125 ppm for 60 min
Sodium hypochlorite (2)*3	100 ppm for 30 s or 10 ppm for 60 min
Benzalkonium chloride*3	0.1% for 30 s or 0.01% for 60 min

*1 More than 90% reduction of RSIV infectivity was observed under the conditions of natural seawater, sunlight, and drying, whereas complete inactivation (>99.9%) was observed under the other conditions. *2 Without organic matter. *3 With organic matter.

Improved approach for detecting KHV by real-time PCR

- We developed an artificially generated plasmid DNA, pKHV-RSIVMCP-1, containing a sequence distinct from that of KHV, which allows differentiation between plasmid DNA contamination and true KHV-derived signals.
- We optimized the conditions for multiplex reactions targeting KHV and the host glucokinase gene assays. The glucokinase assay serves as an internal control to verify the presence and quality of genomic DNA in the reaction mixture.

Kurobe *et al.* submitted

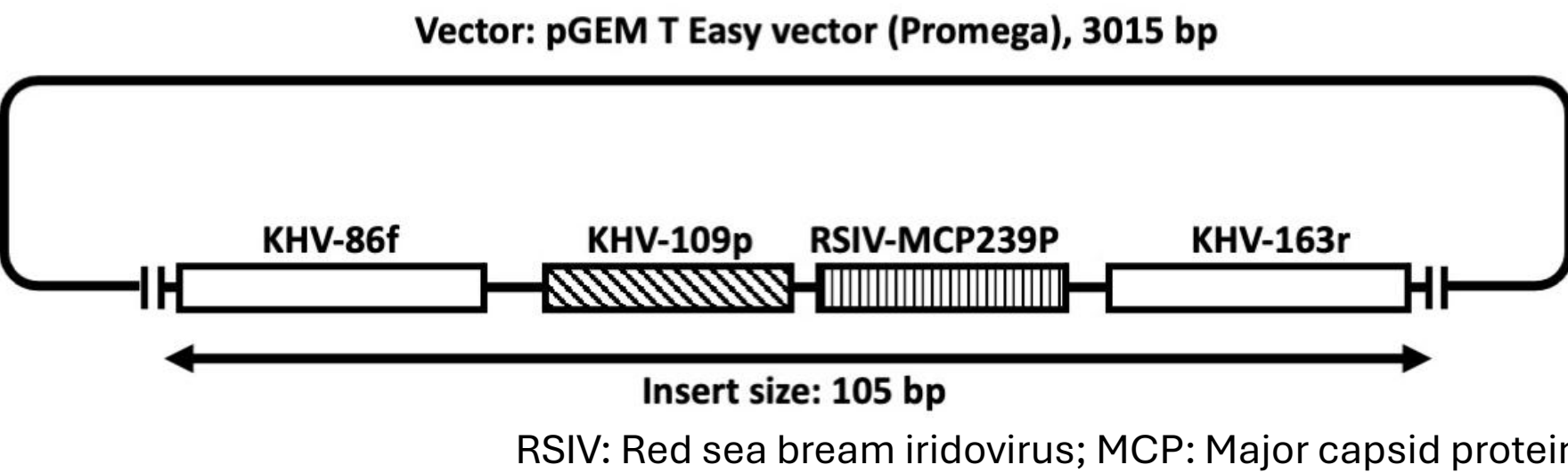


Fig. Schematic illustration of the plasmid DNA, pKHV-RSIVMCP-1

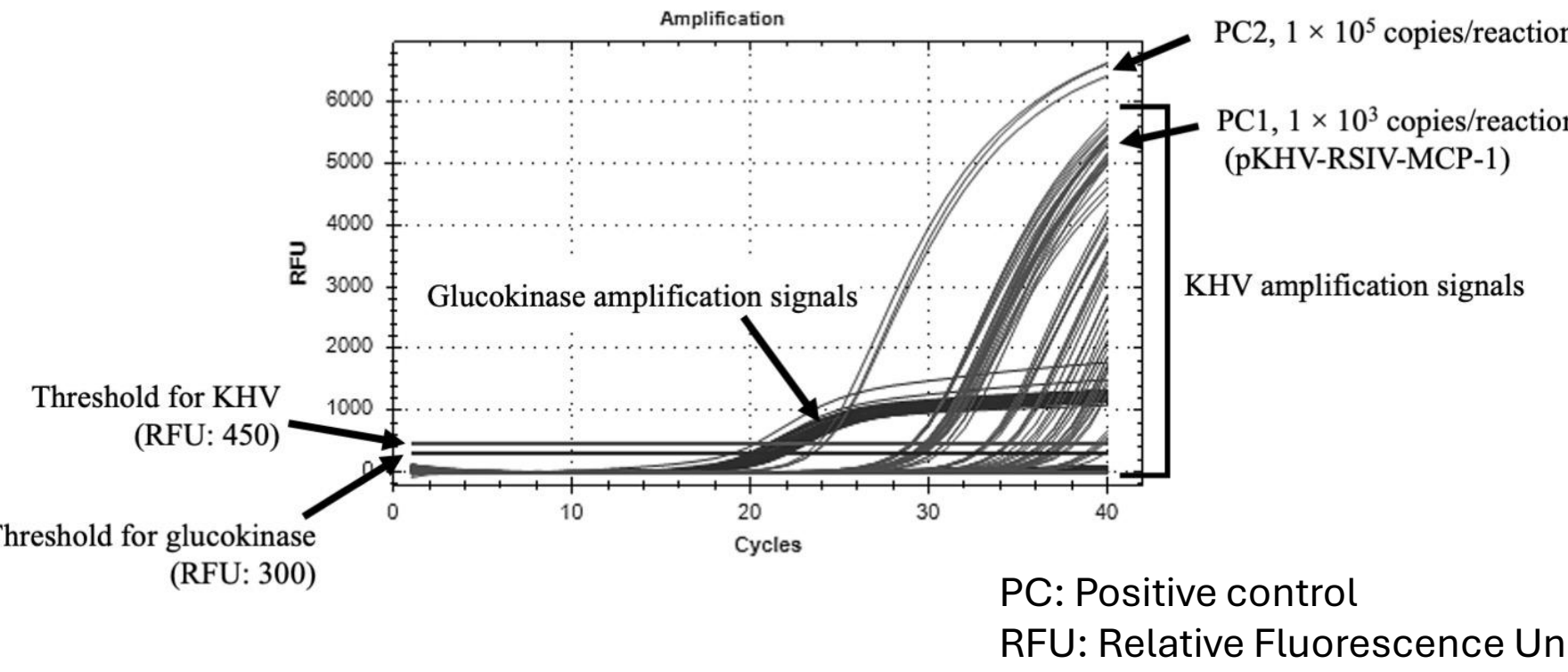
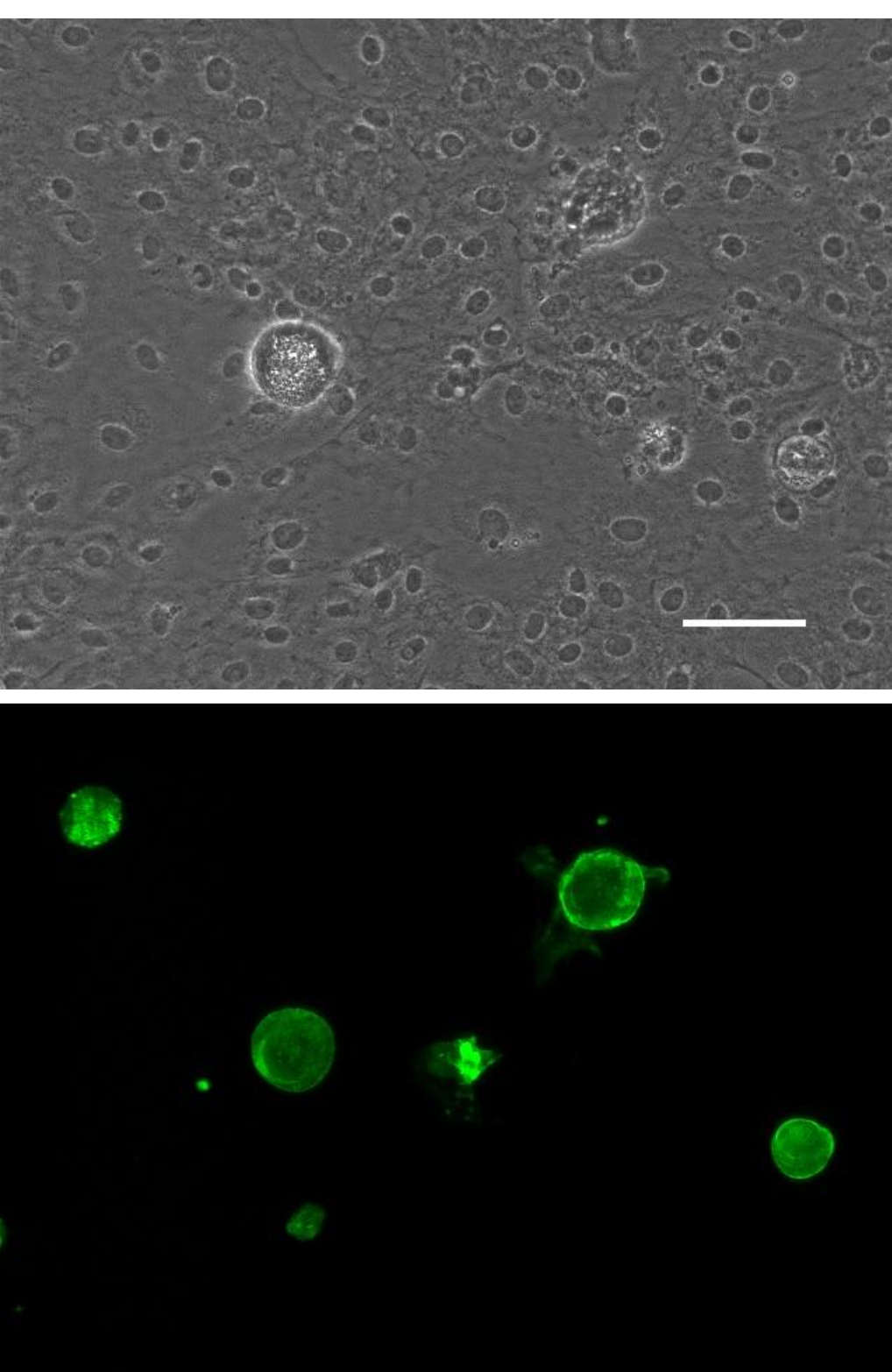


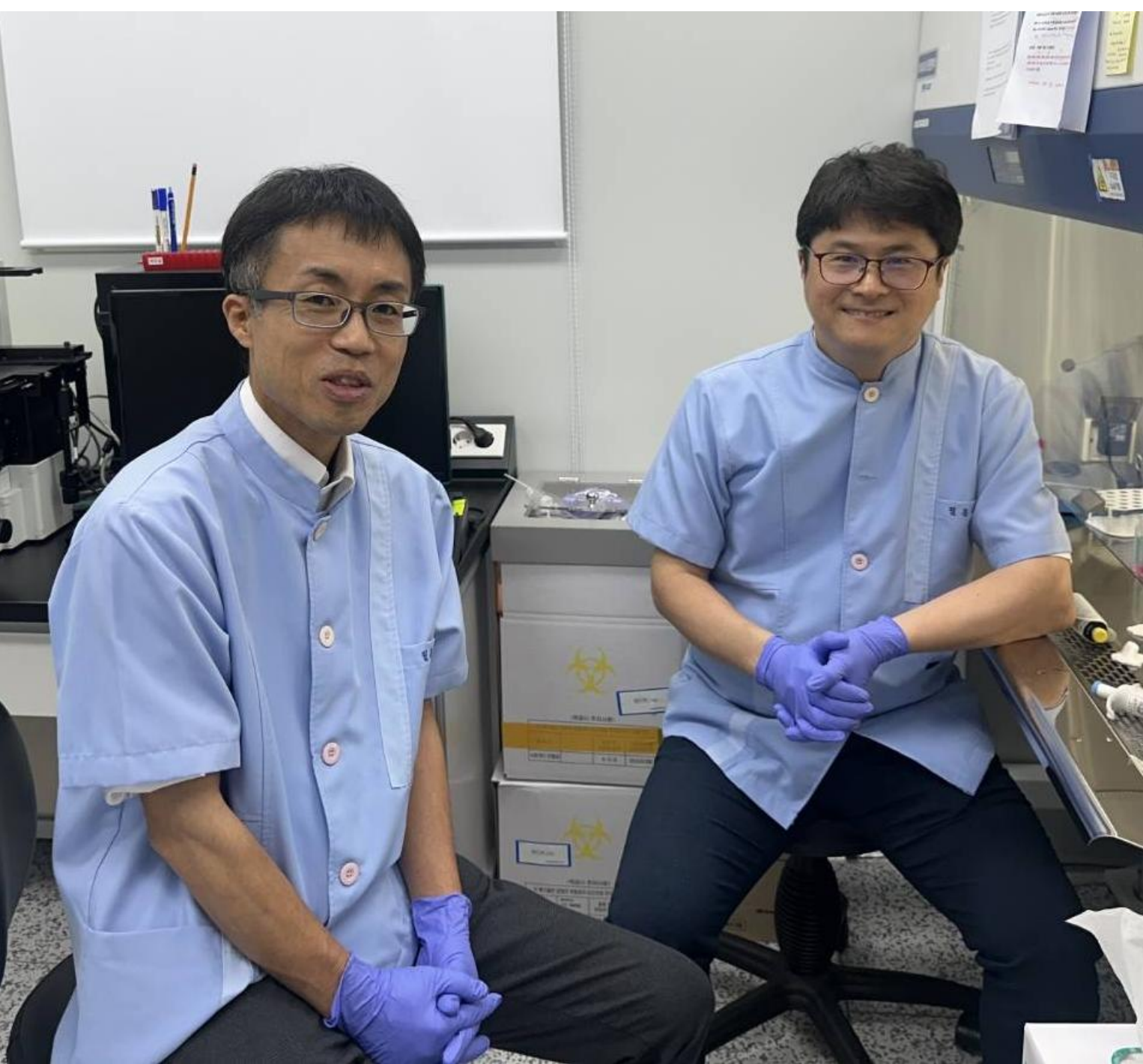
Fig. Amplification plots of the multiplex assay for targeting KHV and the glucokinase gene (or the KHV-Gluc multiplex assay)

4. How can we support Members?

- ✓ Providing disease information (e-mail, web meeting).
- ✓ Providing the materials for diagnosis of *M. pagrus1* and KHV including DNA for PCR, monoclonal antibody, and cell line for virus isolation.
- ✓ Assistance for diagnosis of *M. pagrus1* and KHV infection including confirmatory diagnosis.
- ✓ Research collaboration.
- ✓ Internship or training associated with *M. pagrus1* and KHV research (virus isolation, real-time PCR, eDNA research)



CPE of *M. pagrus1*-infected SKF-9 cell line (upper) and IFAT with monoclonal antibody (lower)



Research collaboration with NIFS (Korea) for isolation of *M. pagrus1* of TRBIV genogroup, June 2025



Hosted an intern from CSIRO ACDP (Australia) for *M. pagrus1* research, June 2025

Contact information

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https://www.fra.go.jp/english/gijutsu/yoshoku/Pathology_Division.html

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