

Introduction on the work of two RLs

- RL for the infection of spring viraemia of carp virus
- RL for the infection of infectious haematopoietic necrosis virus

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General Administration of Customs (GACC)
P. R. China

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01

Introduction of the agency



Animal and Plant Inspection and Quarantine Technical Center
Shenzhen Customs District
General Administration of Customs (GACC)
P. R. China

Undertake quarantine identification, isolation quarantine, species resource identification, research consultation and quarantine risk analysis of entry-exit animals, plants, animal and plant products and other quarantine objects.

Undertake scientific research and technological development and services, provide technical guidance, and participate in the formulation and revision of relevant inspection and quarantine method standards.

Undertake inspection, testing and appraisal services entrusted by governments, institutions and market entities.

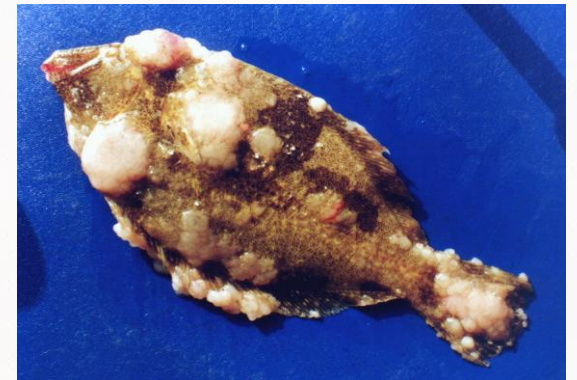


01

Introduction of the agency



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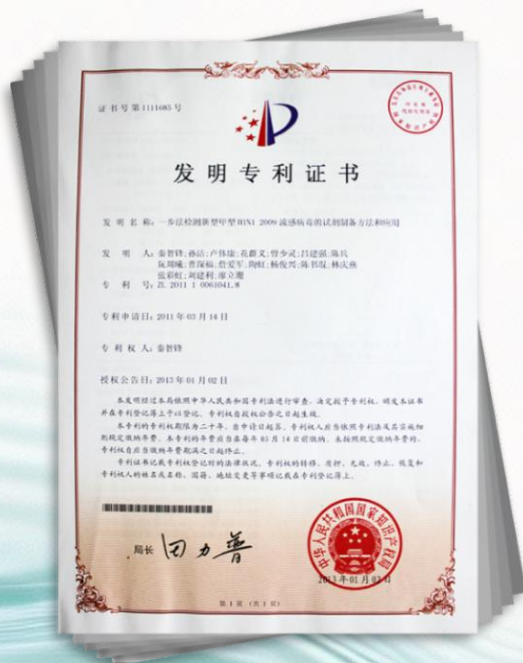


01

Introduction of the agency



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Scientific studies and Standarlization

01

Introduction of the agency



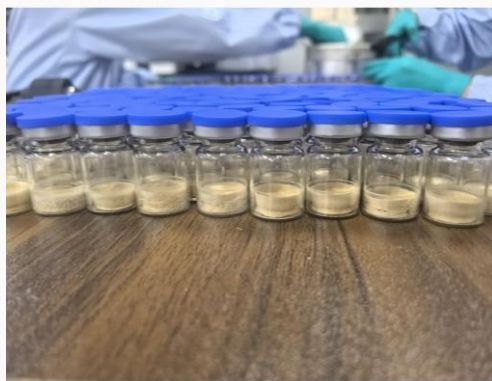
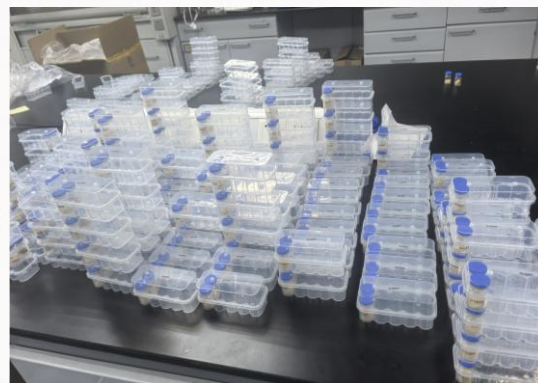
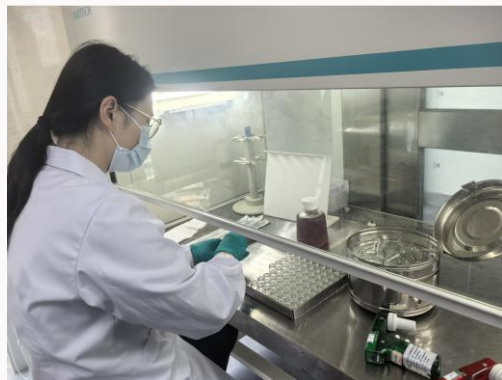
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Training & Communication



02

Surveillance, PT & RT, Training

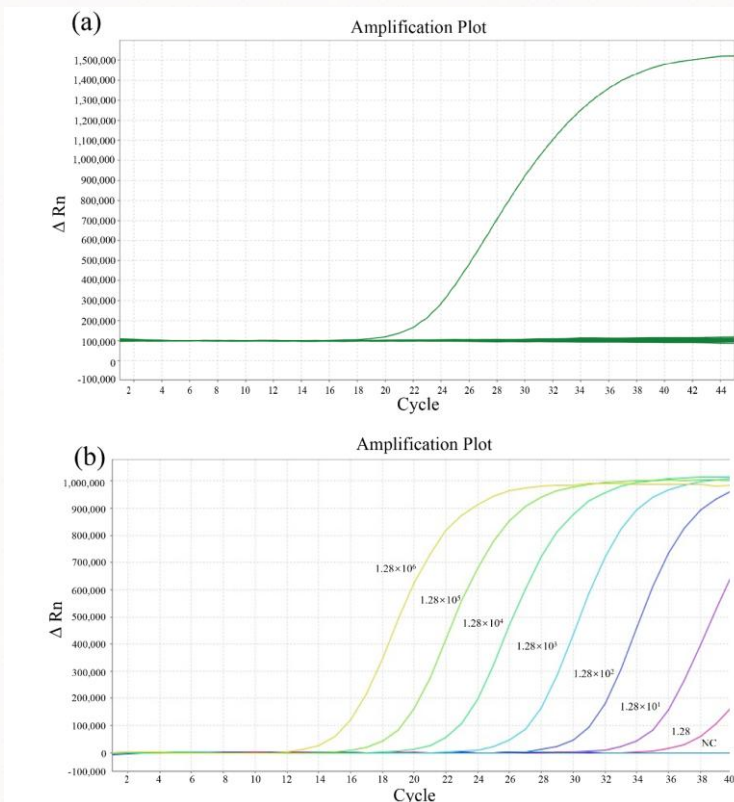


03

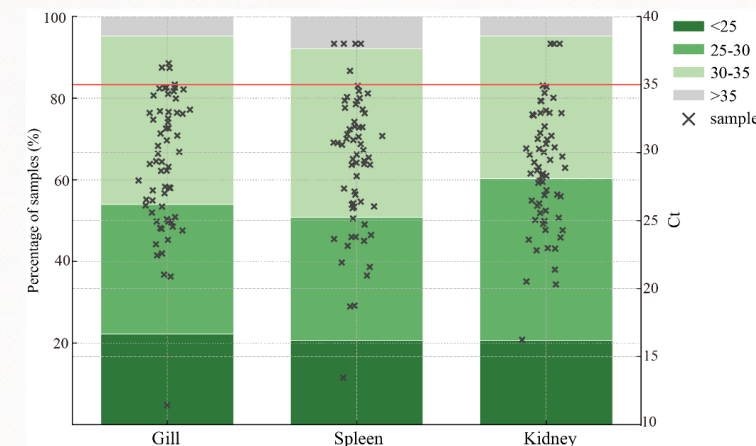
Development and validation of the diagnostic tests

Validation on the real-time RT-PCR test of SVCV

	Forward primer	Probe	Reverse primer
980451	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
980528	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
2-90	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
RHV	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
N1-5	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
PA-7	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
S30	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
M2-78	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
02-131	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
992	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
A36-11	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
A60-70	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
D120	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
E208	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
E232	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
G083	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
G144	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
H243	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
H244	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
L045	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
M031	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
N029	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
P022	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
P106	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
Fijan	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
Bjorklund	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
SVCV-265	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
A1	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
A2	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
SVCV-C1	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
SH140501	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
SH140502	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
SH140503	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
SH140522	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
SH150514	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
SH160901	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
ADC-SVC2016-1	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
ADC-SVC2016-3	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT
ADC-SVC2016-5	TAATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT	5'-GAGACAGAGGTTGAAAGGACATCTGAT	TCATTTGTTGAGACAGAGGTTGAAAGGACATCTGAT



Primer/Probe	sequence (5' to 3')
SVCV Cefas AR-F	5'-CAATGGGAATGAGTGCTTCT-3'
SVCV Cefas AR-R	5'-TGATTGGTTTGAATTGTRTC-3'
SVCV Cefas AR-P	5'-FAM-AGACAGAGGTRAAAGGACATCTGAT-BHQ1-3'



International cooperation with CEFAS, UK (SVC RL)

Sample type	True Positive	False Positive	True Negative	False Negative	Diagnostic Sensitivity	Diagnostic Specificity
Cell-culture isolate	249	0	15	0	100%	100%
Tissue sample	178	0	15	11	94%	100%

03

Development and validation of the diagnostic tests

Validation on the real-time RT-PCR test of IHN

Evaluate the performance of the two WOA-recommended IHNV real-time RT-PCR assays (Purcell et al., 2013 and Hoferer et al., 2019).

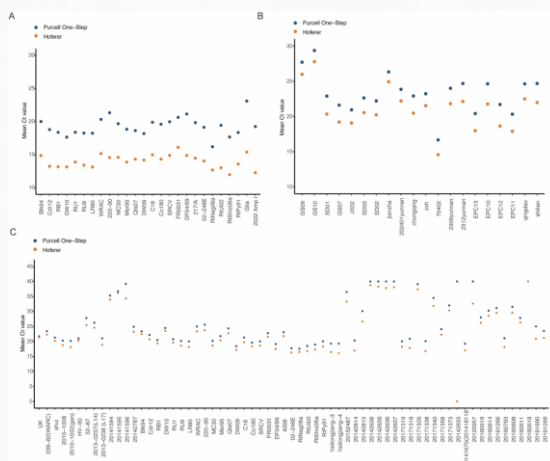


Fig 1. Comparison of Ct values obtained using the Purcell one-step and Hoferer RT-qPCR assays across three participating laboratories.

Mean Ct values for a panel of IHNV isolates tested in triplicate are shown for (A) LAB1, (B) LAB2, and (C) LAB3. For each laboratory, blue circles represent results obtained using the Purcell one-step assay and orange circles represent results obtained using the Hoferer assay.

Conclusion:

1. When both assays were implemented as one-step RT-qPCR methods, all laboratories consistently observed lower Ct values with the Hoferer assay compared with the Purcell assay, indicating superior analytical sensitivity under one-step reaction conditions.
2. When used in its prescribed two-step format, the Purcell assay generally produced lower Ct values than the Hoferer one-step assay for isolates without probe-region mismatches. However, for isolates containing mismatches within the Purcell probe region (e.g., Gila and 2022 Amp I), the Hoferer assay demonstrated superior detection performance.
3. Optimization of the Hoferer one-step protocol (50° C for 30 min reverse transcription) improved assay performance, resulting in Ct values comparable to the Purcell two-step assay while maintaining better detection of isolates with Purcell probe mismatches.

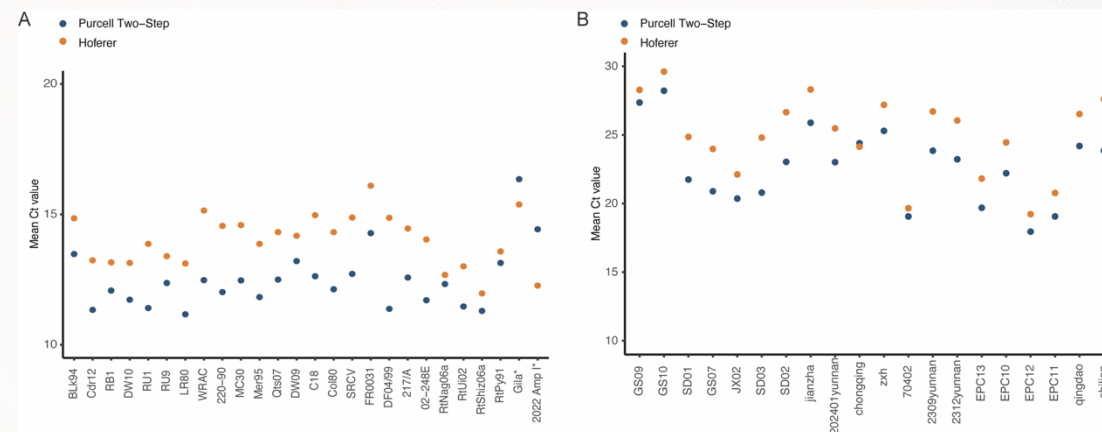


Fig 2. Comparison of Ct values obtained using the Purcell two-step and Hoferer RT-qPCR assays in two participating laboratories.

Mean Ct values for a panel of IHNV isolates tested in triplicate are shown for (A) LAB1 and (B) LAB2. Blue circles represent results generated using the Purcell two-step assay, and orange circles represent results obtained using the Hoferer assay.

Suggestion:

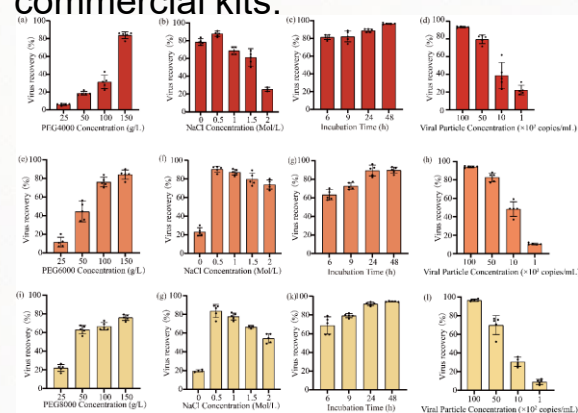
1. It is recommended that both the Purcell and Hoferer IHNV real-time RT-qPCR assays be retained in the WOA Aquatic Manual, as both probe sets demonstrated comprehensive detection across all tested IHNV genotypes.
2. It is further recommended that the optimized cycling conditions for the Hoferer one-step RT-qPCR assay, including a reverse transcription step of 50°C for 30 min, be explicitly specified in the WOA Manual to ensure optimal analytical sensitivity and consistent assay performance.

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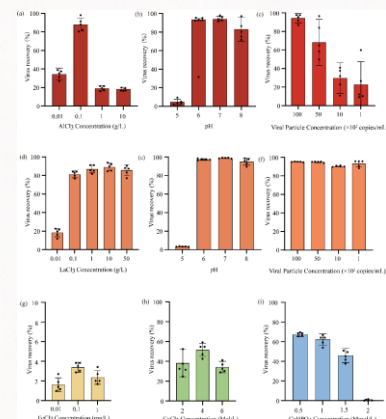
Development and validation of the diagnostic tests

Optimization and Evaluation of an Efficient Enrichment Method for Spring Viraemia of Carp Virus (SVCV) eDNA in Water Environments

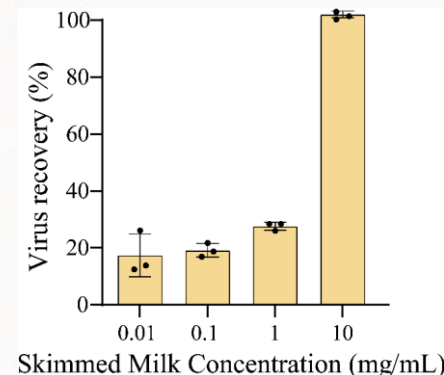
9 enrichment methods, including polyethylene glycol (PEG) precipitation, metal ion flocculation, skimmed milk adsorption, and commercial kits.



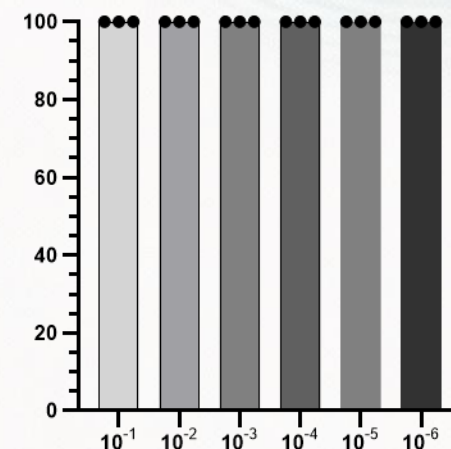
Optimization of PEG enrichment method



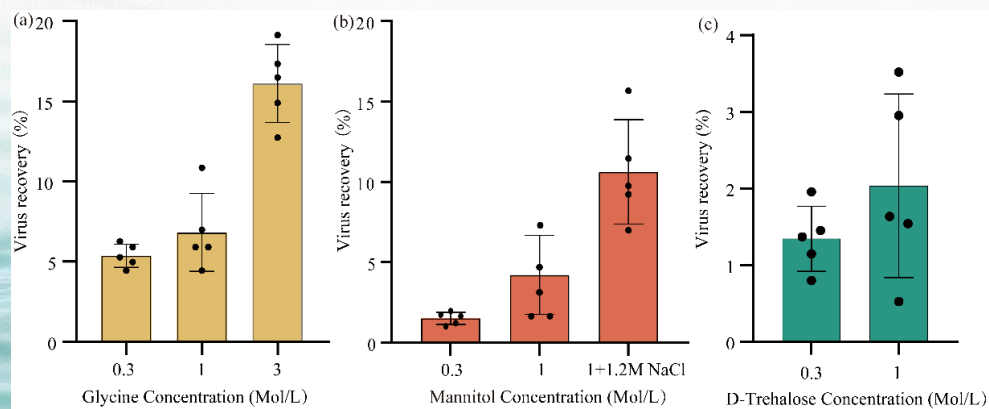
Optimization of metal ion enrichment method



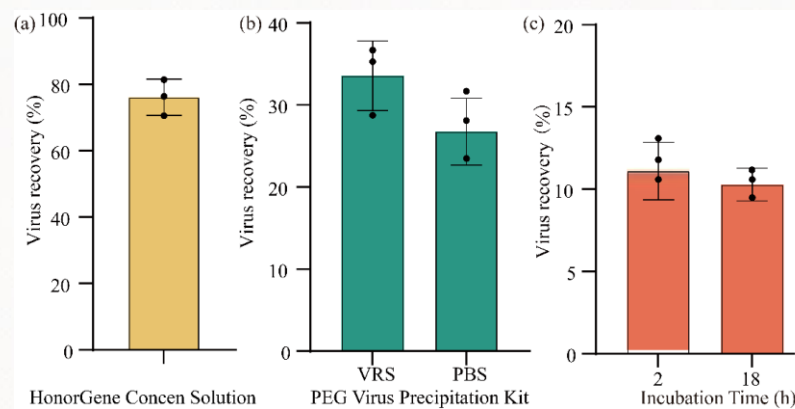
Optimization of skimmed milk powder enrichment method for



Results Diagram of the Sensitivity of Tangential Flow Virus Enrichment



Concentration of enrichment with Mannitol(a), Glycine(b) and D-Trehalose (c)

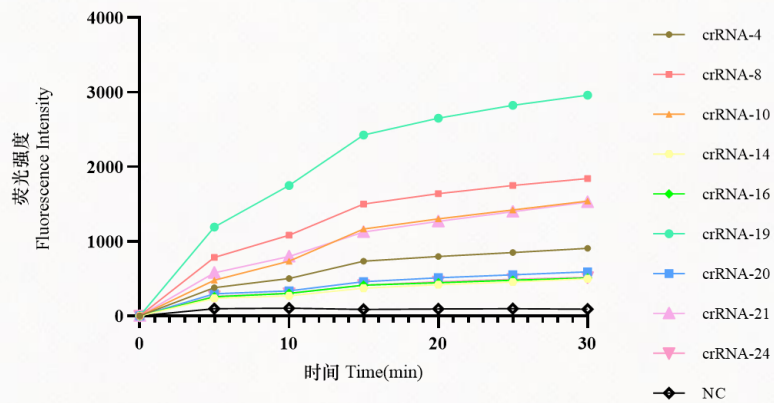


Enrichment Kit Concentration of SVCV

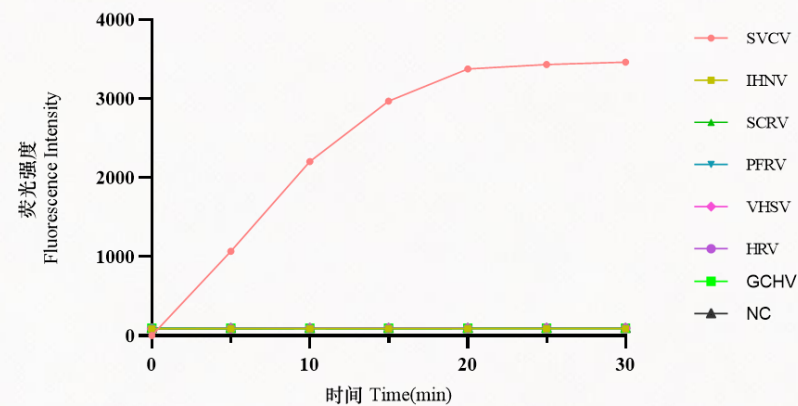
03

Development and validation of the diagnostic tests

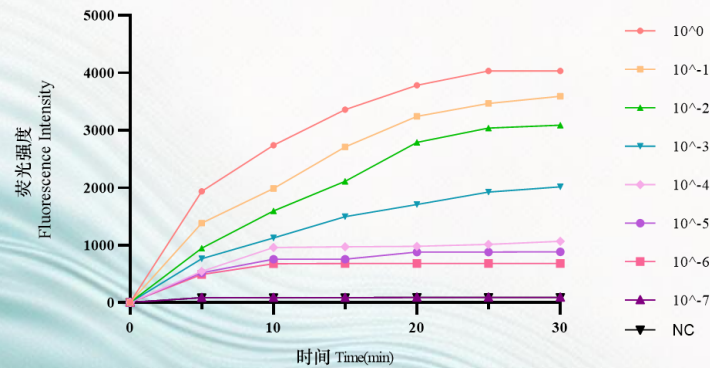
RPA and CRISPR tests on SVCV



Experimental Results of RAA-CRISPR/Cas13a Primer Screening



Results of the Analytical Specificity Test of RAA-CRISPR/Cas13a



Results of the Analytical Sensitivity Test of RAA-CRISPR/Cas13a

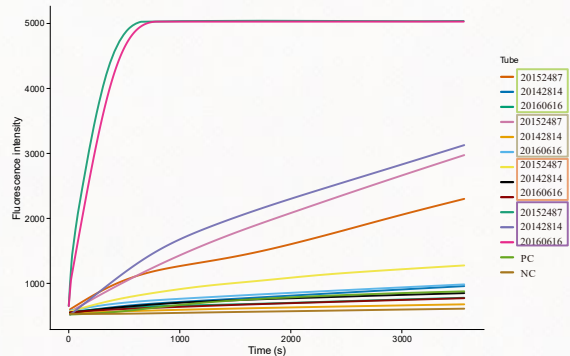
Genogrou p	Numbe r	Virus isolation	Nested RT- PCR	real-time RT- PCR	RAA-CRISPER
Ia	53	P43/N10	P43/N10	P43/N10	P43/N10
Ib	2	P2/N0	P2/N0	P2/N0	P2/N0
Ic	2	P2/N0	P2/N0	P2/N0	P2/N0
Id	3	P3/N0	P3/N0	P3/N0	P3/N0

Detection Results of Actual Samples

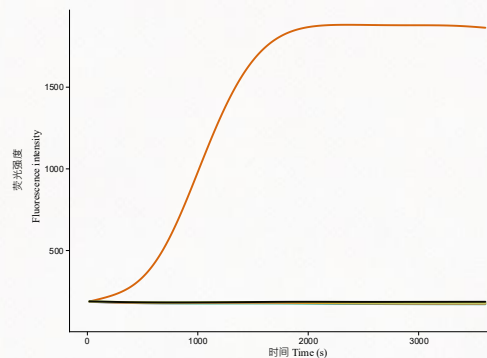
03

Development and validation of the diagnostic tests

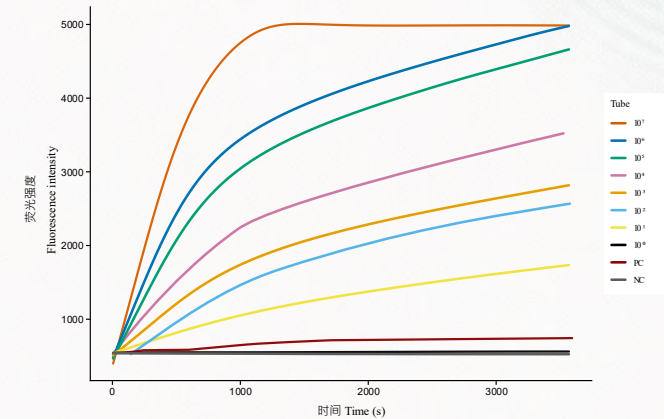
RPA and CRISPR tests on IHN



RAA-CRISPR/Cas13a Primer screening



Analysis Specificity Test



Analysis of Sensitivity Test

Concentration	Intra-assay reproducibility		Inter-assay reproducibility	
	X±SD	CV	X±SD	V
10 ³	1607.27 ± 115.43	7.18	1579.06 ± 34.64	2.19
10 ²	790.00 ± 17.05	2.16	789.55 ± 4.11	0.52
10 ¹	148.97 ± 7.79	5.23	158.37 ± 9.19	5.80

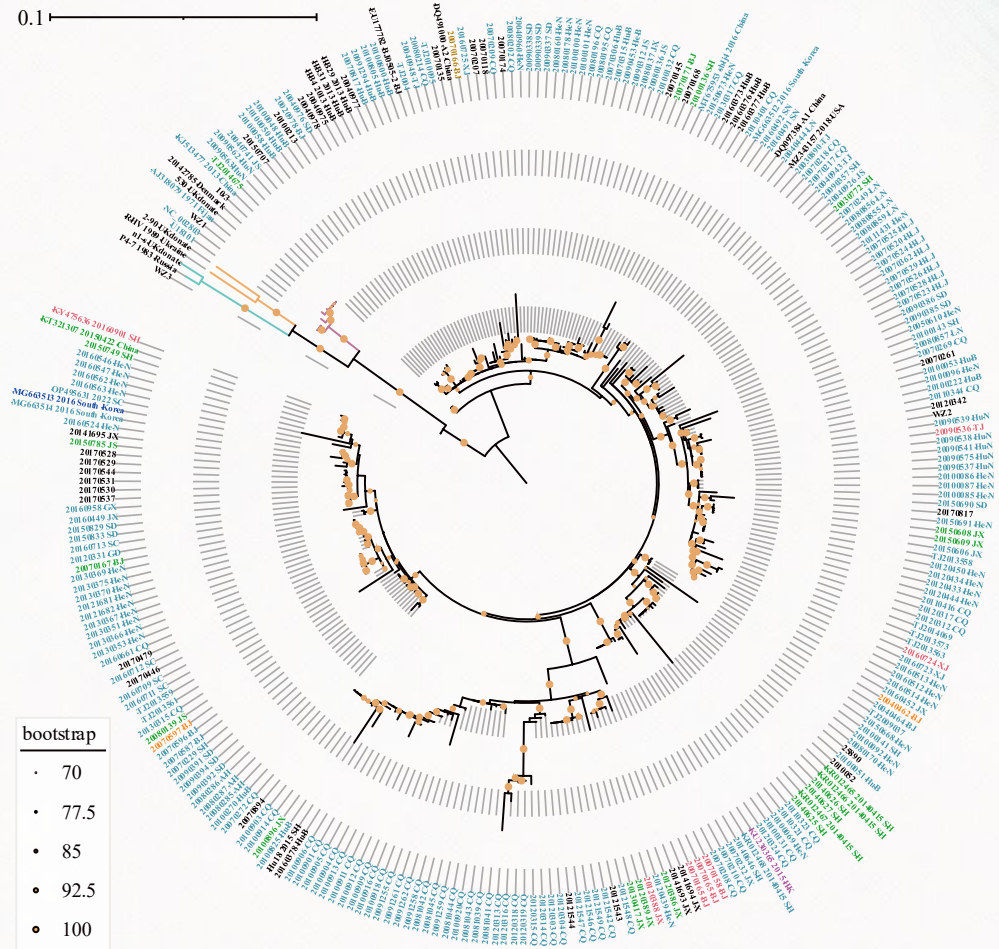
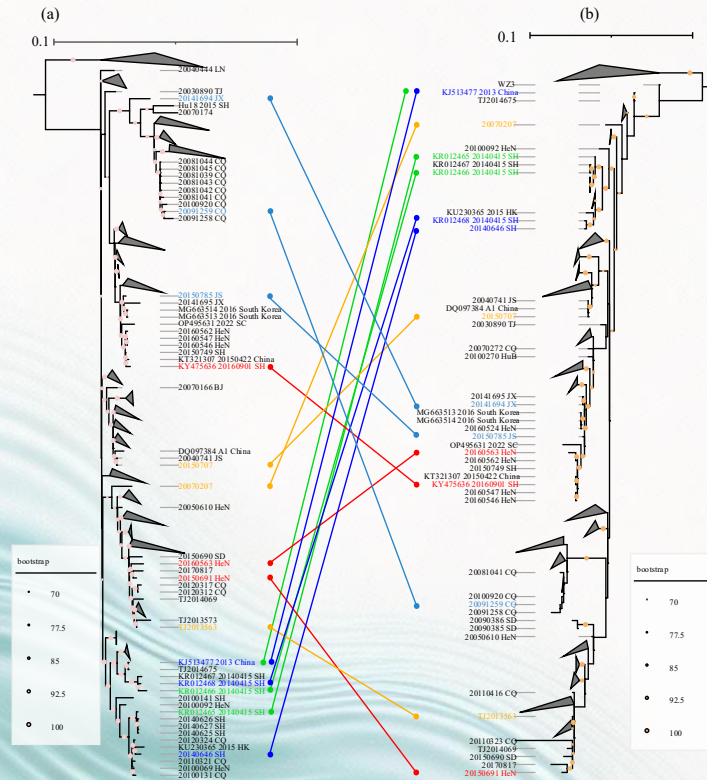
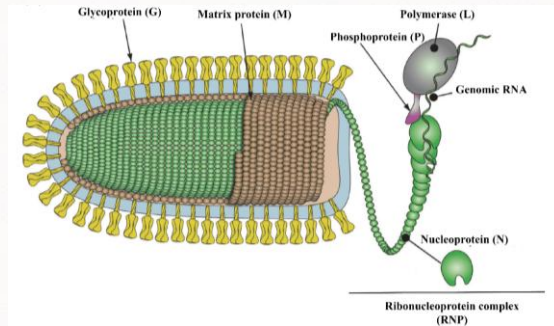
Reproducibility Results

Genotype	Number	Virus isolation	Nested RT-PCR	Real-time RT-PCR	RAA-CRISPR/Cas13a
U	4	P4 / N0	P4 / N0	P4 / N0	P4 / N0
M	6	P6 / N0	P6 / N0	P6 / N0	P6 / N0
L	2	P2 / N0	P2 / N0	P2 / N0	P2 / N0
E	2	P2 / N0	P2 / N0	P2 / N0	P2 / N0
J	34	P26 / N8	P26 / N8	P26 / N8	P26 / N8

Diagnostic sensitivity and diagnostic specificity

04

Epidemiology and molecular Epidemiology studies



Study on the genomic sequences of SVCV in the world

Future



01 Epidemiology

Collection on the epidemiological information
Molecular study and genotyping



02 eDNA

International cooperation
Optimization
Practice
Standardization



03 Validation on new tests

Validation and recommendation
Reference substances preparation and distribution



04 Prevention and control measure

Medicine
Vaccine
Biosecurity measure (Compartmentalization)

Thanks for your attention



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