IQ2000™ SVCV

Instruction Manual

Spring Viremia of Carp

in-vitro use only

No contagious materials included

2014/07

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I. Introduction

Spring Viremia of Carp (SVC) is a contagious viral disease affecting cyprinid fish. Not only common carp (*Cyprinus carpio*), infections have also been reported in koi carp (*Cyprinus carpio koi*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalamicthys molitrix*), bighead carp (*Aristicthys nobili*s), crucian carp (*Carassius carassius*), golden fish (*Carassius auratus*), tench (*Tinca tinca*), orfe (*Leuciscus idus*), and sheatfish/European catfish/wels (*Silurus glanis*).

Until today, the SVC has been reported in Europe including UK, Russia, Belarus, Georgia, Lithuania, Moldova, Ukraine, Middle East, China, USA and Brazil. According to the statistics, in Europe, 10-15% of yearly carps are lost to SVC each year and the mortality has reached 70% in one-year-old carps.

Spring Viremia of Carp is caused by the spring viremia of carp virus (SVCV). It belongs to Rhabdoviridae and is a bullet-shaped RNA virus. Fish can carry SVCV with or without symptoms. The virus enters the fish most often through the gills and replicates in gill epithelium. Because of the longevity of the virus in water, mud, or following desiccation, transmission is thought to be by direct contact or through the water. SVCV isolates can be divided into four genetic groups. And the primer sets in the IQ2000™ SVCV Detection and Prevention System was designed to detect the genogroup IA which originate from Asia.

The IQ2000™ SVCV Detection and Prevention System has adopted the design of nested PCR and also inherited the reliability, sensitivity and experience from a range of IQ2000™ virus diagnostic kits for shrimp and fish virus disease. Its unique semi-quantitative function provides information of virus infection levels which could be used for further understanding on SVCV.

II. System Components

1. **RNA Extraction Solution** (200 reactions/kit) 100 ml/btl
   store at 4℃
   DEPC ddH_2O 100 ml/btl
   store at –20℃

2. SVCV specific sequence amplification kit (200 reactions/kit):
   store at –20℃
   - **RT-PCR PreMix** 4 vials 420 μl/vial
     includes reaction buffer, dNTPs, and SVCV specific primers
   - **Nested PCR PreMix** 4 vials 840 μl/vial
     includes reaction buffer, dNTPs, and SVCV specific primers
   - **P(+) standard** 1 vial 100 μl/vial
     10^4 copies/μl plasmids containing SVCV partial sequence
   - **Yeast tRNA (40 ng/μl)** 1 vial 500 μl/vial
   - **IQzyme DNA polymerase (2 U/μl)** 1 vial 360 μl/vial
   - **RT Enzyme Mix** 1 vial 120 μl/vial
   - **6X loading dye** 1 vial 1,500 μl/vial
   - **DNA molecular weight marker** 1 vial 100 μl/vial
     848 bp, 630 bp & 333 bp

III. Equipment and reagents required but NOT provided

1. Thermal cycler with sample block which fits 0.2-ml thin-walled tube
2. High-speed bench top microcentrifuge (12,000 x g; 12,000 rpm, r = 5~8 cm)
3. Electrophoresis apparatus
4. UV transilluminator
5. Vortex mixer
6. Heating block
7. Micropipette
8. Polaroid camera or digital photo system
9. Chloroform
10. Isopropanol
11. 75% ethanol
12. Ethidium bromide
13. TAE or TBE electrophoresis buffer
14. Agarose

IV. Detection Limit and Sensitivity

This detection system reaches different detection limits for different sample types tested. The following table lists some common samples tested. Based on the knowledge of viral distribution in fish, gill, spleen and kidney sample is recommended for screening.

Specimen	Test Quantity	Detection limit (copies/reaction)
SVCV DNA plasmid	5 copies/μl	10
In-vitro transcribed RNA	20 copies	20
Gill	Small piece	20
Spleen	20 mg	20
Kidney	20 mg	20

Base on the table above, users have to know that negative test result mean the specimen is not infected or virus titers in the specimen are lower than the detection limit. All the test results listed in the table were obtained by following the standard procedure and reagents described in this manual. We do not guarantee RNA extracted by RNA extraction reagents from other manufacturers would be compatible with our detection system.

V. Sample preparation and RNA extraction

1. RNA extraction procedure

   a. Place sample into a 1.5 ml tube that contains 500 μl RNA Extraction solution.
   b. Grind the sample in the tube with a disposable grinder, stand in room temperature for 5 minutes.
   c. Add 100 μl of $CHCl_3$ then vortex 20 seconds. Sit in room temperature for 3 minutes, then centrifuge it at 12,000 x g (12,000 rpm, r = 5~8 cm) for 15 minutes.
   d. Transfer 200 μl of the upper clear aqueous phase to a new 0.5 ml tube with 200 μl 2-propanol (isopropanol).
   e. Vortex briefly, centrifuge at 12,000 x g for 10 minutes, then decant the isopropanol.
   f. Wash the pellet with 500 μl of 75% ethanol, then spin down 5 minutes by 7,500 x g to recover RNA pellet, then decant the ethanol and dry the pellet.
   g. Dissolve the pellet with DEPC ddH_2O.

2. RNA dissolution

a. The amounts of RNA from different sample sources vary, therefore the concentration of RNA should be adjusted by dissolving the RNA pellet in different volumes of DEPC ddH_2O.

SAMPLE SOURCE	VOLUME
Gill	100 μl
Spleen	500 μl
Kidney	500 μl

b. Please fine tune the volume of DEPC ddH_2O according to the real recovery efficiency.

VI. Amplification Protocol

The following amplification conditions apply to 0.2-ml thin-wall tubes or 96-well plates. Before executing the PCR procedures, please make sure the hot start function of the machine has already been disable.

1. Reagents preparation:
   a. RT-PCR reaction reagent mixture: 8 µl/reaction
      Mix the following:

RT-PCR PreMix	7.0 µl
IQzyme DNA Polymerase (2 U/µl)	0.5 µl
RT Enzyme Mix	0.5 µl

   b. Nested PCR reaction reagent mixture: 15 µl/reaction
      Mix the following:

Nested PCR PreMix	14 µl
IQzyme DNA Polymerase (2 U/µl)	1 µl

2. Reaction condition (Uni-IQ RT-PCR profile):
   a. RT-PCR reaction profile:
      42℃, 30 min; 94℃, 2 min; then
      94℃, 20 sec; 62℃, 20 sec; 72℃, 30 sec, repeat 15 cycles, then add
      72℃, 30 sec; 20℃, 30 sec at the end of the final cycle.
   b. Nested PCR reaction profile:
      94℃, 20 sec; 62℃, 20 sec; 72℃, 30 sec, repeat 30 cycles, then add
      72℃, 30 sec; 20℃, 30 sec at the end of the final cycle.

3. Reaction procedure:

   a. Prepare RT-PCR and nested PCR reaction reagent mixtures required according the sample number. For each reaction mixture preparation, user also needs to include 3 positive standards (10^3, 10^2 and 10^1) and 1 negative control (ddH_2O or Yeast tRNA).
   b. Pipette 8 µl of RT-PCR reaction reagent mixture into each 0.2 ml reaction tube with proper label.
   c. Add 2 µl of the extracted sample RNA or standard* into each reaction mixture.
   d. Perform RT-PCR reaction profile.
   e. Add 15 µl of nested PCR reaction reagent mixture to each tube after RT-PCR reaction is completed.
   f. Perform nested PCR reaction profile.
   g. After nested PCR reaction is completed, add 5 µl of 6X loading dye to each reaction tube and mix well.
   h. Sample is ready for electrophoresis.

*The inclusion of 10 copies/µl standard is highly recommended for every batch of test to monitor the sensitivity of the reaction. We also recommend to use Yeast tRNA to dilute the positive standards, which can be kept at –20℃ for a week afterwards.

VII. Electrophoresis

1. Agarose gel preparation

   a. First, decide a buffer system of electrophoresis between TAE and TBE.

   b. Then, dilute the buffer to 1X operation concentration to run electrophoresis and prepare agarose gels. Note that use the same buffer to run electrophoresis and prepare agarose gels.

   c. A 2% agarose gel is recommended for electrophoresis. To prepare 2% agarose gel, add 2 g agarose into a wide mouth glass bottle or flask with 100 ml electrophoresis buffer.

   d. Heat the mixture until it becomes hyaline without any agarose particles. Heating can be done by using an alcohol lamp, gas lamp or heat plate. A microwave oven can also be used. To avoid agarose solution from boiling over, heat the solution in a large glass container (twice the solution volume).

   e. Cool down the clear agarose solution at room temperature until it reaches about 50℃. Slowly pour the gel into the gel box. The volume of the agarose solution varies according to the size of the gel box. Generally speaking, the height of agarose gel only has to reach about 0.3~0.5 cm and no more than 0.8 cm.

      After agarose gel is completely set, carefully remove the plastic comb and blockers while holding on the top of the comb from both sides. This agarose gel is ready for electrophoresis and shouldn't be left at room temperature for longer than 4 hours.

2. Electrophoresis

a. a. Lay the agarose gel inside the gel box. DNA molecules move toward (+) pole because they are negatively charged.

b. Add 1X electrophoresis buffer into the gel box until the buffer just covers the gel.

c. Load 5 μl each of the "PCR product-loading dye mixture" into each well. The mixture will sink to the bottom of the wells because its density is higher than the electrophoresis buffer. This step should be handled carefully in order to avoid spillover and cross-well contamination.

d. The DNA Molecular Weight Markers serve as reference for PCR product size. DNA markers are required for every electrophoresis run. About 5 μl of DNA markers are recommended.

e. After all the samples are loaded, connect the gel box with power supply. Switch on the power supply and run electrophoresis at constant voltage between 100V~150V.

f. The loading dye in the kit contains 2 dyes: Bromphenol Blue is deep blue; and Xylene Cyanol is light blue. When the deep blue dye approaches 1/2 to 2/3 of the gel, stop the electrophoresis. Then, remove the gel from the gel box to proceed to the EtBr staining procedures.

g. To avoid PCR product contamination, DO NOT reuse the gel electrophoresis buffer unless several gels will be run in the same day. When the electrophoresis is finished, rinse the gel box with plenty of water.

3. Gel staining and data assay

a. Ethidium Bromide (EtBr) is usually prepared as a 10 mg/ml stock solution. This solution should be stored in an amber container because EtBr is a light degradable chemical. Note that EtBr is a known carcinogen, the use of protective suit, gloves, and goggles are highly recommended.

b. Dilute the 10 mg/ml stock solution 20,000 folds (*e.g.* add 5 μl of the above stock solution into 100 ml distilled water to prepare the staining solution.).

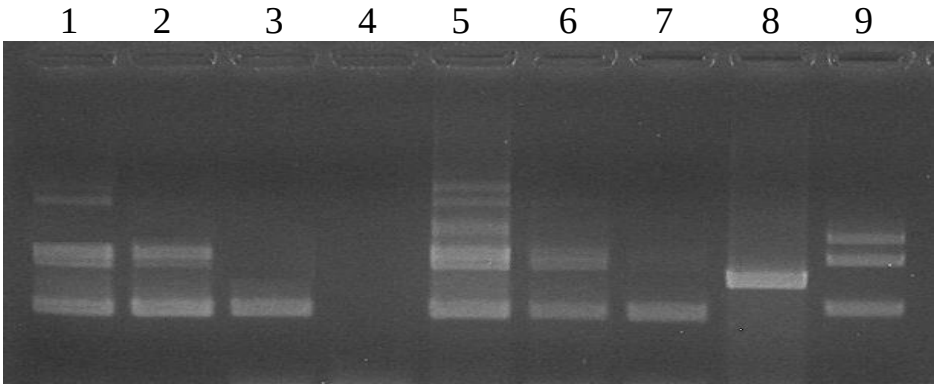
c. Pour the above staining solution into a plastic tray or zip-lock bag with the gels from step 2. The solution must cover the whole gel.

d. Shake lightly at room temperature for 10 minutes. Then, distain the gel with distilled water in another plastic tray for another 10 minutes to eliminate the background.

e. Lay the gel on a UV transilluminator to record the final result.

VIII. Diagnosis

1. An example of the results is shown and explained below:



Lane 1: SVCV P(+) standard, 2,000 copies/reaction
Lane 2: SVCV P(+) standard, 200 copies/reaction
Lane 3: SVCV P(+) standard, 20 copies/reaction
Lane 4: Negative control (Yeast tRNA)
Lane 5: Sample of severe SVCV infection
Lane 6: Sample of moderate SVCV infection
Lane 7: Sample of light SVCV infection
Lane 8: SVCV negative sample
Lane 9: Molecular weight markers, 848 bp, 630 bp, 333 bp

2. Negative samples will show only the 471 bp band, which is the product of a house keeping gene (internal control).

3. Interpretation:
   a. Bands of 295 bp and/or 580 bp are present: P(+)
   b. Only a band of 471 bp is present: N(-)

4. Each experiment requires positive and negative controls. The absence of the 295 bp band in the 10^2 reaction implies that the PCR reaction failed or other possibilities. On the other hand, the present of the 295 bp band in the negative control reaction means that contamination has occurred. For more information, please refer to the Troubleshooting section or contact GeneReach.

IX. Troubleshooting

Observation or Problems	Possible causes	Comments or solution
Faint bands or no bands are detected after staining.	1. EtBr has been degraded.	1. Prepare new EtBr solution or extend staining time.
	2. UV light was not turned on.	2. Check UV transilluminator.
	3. Background is too strong.	3. Soak gel in water at 4℃ for another 30 minutes.
	4. Agarose gel is too thick.	4. Check thickness of gel. If gel is thicker than 0.8 cm, prepare a gel of proper thickness and run the electrophoresis again.
Band of expected sizes in positive standard but no bands in marker lane.	Markers were degraded or under-loaded.	Use markers from a different tube or increase loading volume.
Expected bands in marker lane but no bands in P(+) lane.	1. PCR failed.	1. Check reagent mixture preparation record and PCR cycle profile setting.
	2. Enzyme was not added.	2. Add enzyme.
	3. P(+) was degraded.	3. Prepare new P(+).
Bands in high P(+) (10^3) lane but no bands in P(+) (10^2) and (10).	1. P(+) was degraded.	1. Prepare new P(+).
	2. P(+) dilution was done in H_2O instead of tRNA.	2. Prepare new P(+) using tRNA as diluent.
	3. 10^3 standard was degraded.	3. Replace 10^3 standard.
	4. Low enzyme activity.	4. Check expiration date and storage condition of enzyme, or replace enzyme.

Observation or Problems	Possible causes	Comments or solution
Negative (-) control shows a band at 284 bp.	1. Micropipette contamination.	1. Disassemble and clean up pipette. We recommend using aerosol-free tips. Also, a separate pipette should be reserved for PCR product pipetting.
	2. Reagent contamination.	2. Replace reagents.
	3. Lab contamination.	3. Consult with GeneReach for lab clean up
P(+) control and N(-) produced expected banding, but known-infected sample produced no bands.	1. RNA extraction failed.	1. Check RNA extraction procedure
	2. RNA quality is poor or RNA concentration is too high.	2. Check OD 260/280 ratio. Normally, this ratio should be 1.8 to 2.0.
	3. PCR inhibitor.	3. Spike 10^3 P(+) standard into a sample reaction for a parallel PCR reaction. If the one with 10^3 P(+) produced expected bands, then inhibition could be ruled out. No products are produced from 10^3 P(+) suggests the presence of PCR inhibition. Users need to redo RNA extraction.

X. Reference:

1. Ahne W, Bjorklund HV, Essbauer S, Fijan N, Kurath G, Winton JR. Spring viremia of carp (SVC). Dis Aquat Organ. 2002; 52: 261-72.
2. Petty, Barbara D., Allen C. Riggs, RuthEllen Klinger, Roy Yanong, and Ruth Franscis-Floyd. Spring viremia of Carp. University of Florida, Extension. Fact Sheet VM-142.
3. Stone D. M., W. Ahne, K. L. Denham, P. F. Dixon, C. T.-Y. Liu, A. M. Sheppard, G. R. Taylor, K. Way. Nucleotide sequence analysis of the glycoprotein gene of putative spring viraemia of carp virus and pike fry rhabdovirus isolates reveals four genogroups. Dis Aquat Organ. 2003; 53: 203-210.
4. Warg JV., Dikkeboom AL., Goodwin AE., Snekvik K., Whitney J. Comparison of multiple genes of spring viremia of carp viruses isolated in the United States. Virus Gene. 2007; 35:87-95.
5. SPRING VIRAEMIA OF CARP, Manual of Diagnostic Tests for Aquatic Animals 2009, OIE.

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