研究用

コスモ・バイオ株式会社

VHSV ELISA KIT

BIO K 272/1 - BIO K 272/2

Viral haemorrhagic septicaemia (VHS) is a disease of farmed rainbow trout, farmed turbot, farmed Japanese flounder as well as several wild freshwater and marine species caused by VHSV rhabdovirus. Disease generally occurs at temperature between +4°C and +14°C. At water temperature between +15°C and +18°C, the disease generally takes a short course with a modest accumulated mortality. Disease rarely occurs at higher temperatures. VHS outbreaks occur during all seasons, but are most common in spring when water temperatures are rising or fluctuating.

The clinical signs of the disease are high mortality (which can reach up to 100% in fry), especially during the young trout's first winter. The subjects exhibit lethargy, melanosis and exophthalmia. The paleness of their gills reflects their anaemic condition. An autopsy will reveal the presence of numerous sites of haemorrhages in the viscera and muscle mass, distended abdomen due to oedema in the peritoneal cavity. VHS can also occur in a nervous form, characterised by severe abnormal swimming behaviour, such as constant flashing and/or spiralling. It is practically impossible to distinguish VHS from infectious haematopoietic necrosis (IHN) - another Salmonidae viral infection likewise caused by a rhabdovirus - on the basis of clinical examination alone. A differential diagnosis must thus be performed in a laboratory.

The VHSV ELISA test confirms the virus's growth on a susceptible cell line.

Reliable Results

The use of monoclonal antibody as conjugate ensures excellent specificity and very reliable results.

Ease-of-Use

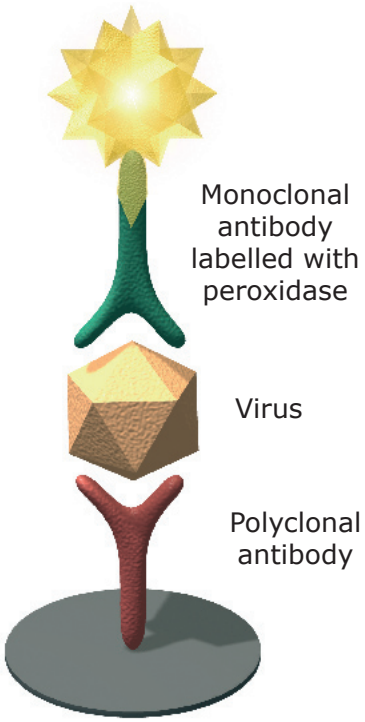
Minimal hands-on-time
Room temperature incubation
Results available in 140 minutes.

Flexibility

Results can be read visually or spectrophotometrically.

EIA Procedure

1- Microplate coated with polyclonal antibodies
2- Add samples and positive control.
Incubate 1 hour at 21°C +/- 3°C
Wash
3- Add conjugates.
Incubate 1 hour at 21°C +/- 3°C .
Wash
4- Add chromogen (TMB)
Wait 10 minutes.
Add stop solution.
Read at 450 nm



Bio-X Diagnostics
Site du complexe des Postes 49, rue Joseph Wauters BE-5580 Jemelle BELGIUM (Europe)
Tel: +32 (0)84.32.23.77 Fax: +32 (0)84.31.52.63 E-mail: info@biox.com http://www.biox.com

Example of results

Nationales Referenzlabor für Fischkrankheiten,
im Institut für Infektionmedizin
Insel Riems, 19.09.06

Cell culture
RTG-2, CCLV, Rainbow trout gonade
FHM, CCLV, Feed head minnow
EPC, CCLV, Epitheilioma papulosum cyprini

Viruses Names	Strains
VHS1014	strain TUV, Denmark
VHS1015	Riemser VHS-Vakzine
VHS1016	reference strain Klapmølle (Denmark)
VHS1017	reference strain 23.75 (France)
VHS1018	Ö62/96, Österreich
VHS1019	Strain 07/71, France
VHS1020	reference strain Voldbjerg (Denmark)
VHS1022	Laborstamm Fi13 (ENZMANN)
VHS1034	Laborstamm Fi13 (ENZMANN)
VHS1036	Isolat 05/00, Deutschland
VHS1037	marine isolate IP8 (herring)
VHS1038	Isolat DF72/94 (Germany)
VHS-Pool 1039/40/41	Laborstamm Fi13 (ENZMANN),TV-Infektionsvirus
SVC1231	Isolat VF, Deutschland
SVC1232	Isolat
SVC1233	Isolat DF 17/00, Deutschland
SVC1234	Isolat DF 17/00 (Germany)
SVC1238	reference strain RC 56/70 (FIJAN)
IHN252	Isolate 4008, Italy
IHN259	Isolat Df 04/99, Deutschland
IHN260	Isolat KINKELIN; France
IHN274	Isolate 4008, Italy
IHN280	isolate 233 (Germany)
IPN449	reference strain Abild, Ab (Denmark)
IPN450	reference strain Spjarup, Sp (Denmark)
IPN451	Birnavirus II, CRL Aarhus, Denmark
IPN452	reference strain Abild, Ab (Denmark)
IPN453	reference strain VR299 (U.S.A.)
IPN455	reference strain VR299 (U.S.A.)
IPN457	reference strain Abild (Denmark)
IPN459	reference strain Spjarup, Sp (Denmark IPN455

Bio-X Diagnostics
Site du complexe des Postes 49, rue Joseph Wauters BE-5580 Jemelle BELGIUM (Europe)
Tel: +32 (0)84.32.23.77 Fax: +32 (0)84.31.52.63 E-mail: info@biox.com http://www.biox.com

Sensitivity VHSV Batch: VHS06D24 Datum 21.06.06

Virus Titre	VHS1017 (6.5/ml)	VHS1018 (6.0/ml)	VHS1019 (7.5/ml)	VHS1020 (5.5/ml)	VHS1036 (NT/ml)	VHS1037 (5.5/ml)	VHS1038 (6.5/ml)	VHS-pool 1039/40/41 (7.75/ml)
Dilution								
10^0	0.686 +	0.332+	1.240+	0.253+	1.399+	0.223+	0.914+	1.801+
10^1	0.165+	0.127-	1.240+	0.121-	0.249+	0.077-	0.228+	0.470+
10^2	0.084-	0.065-	0.077-	0.084-	0.077-	0.077-	0.068-	0.08-
10^3	0.103-	0.071-	0.087-	0.029-	0.083-	0.059-	0.117-	0.046-
10^4	0.073-	0.087-	0.001-	0.069-	0.095-	0.043-	0.061-	0.051-
10^5		0.122-	0.058-	0.07-	0.027-	0.049-	-0.037-	0.071-
Titre	5.5/ml	6.0/ml	6.5/ml	5.5/ml	?/ml	5.5/ml	5.5/ml	6.75/ml
Positive control from the kit: 2.180								

Specificity VHSV Batch: VHS06D24 Datum 04.07.06

Strains (pool)	Dilution	OD	Status
IHN280	undiluted	0.006	negative
IHN274	undiluted	0.146	negative
IHN260	undiluted	0.108	negative
IHN259	undiluted	0.080	negative
IHN252	undiluted	0.033	negative
SVC1238	undiluted	0.065	negative
SVC1233	undiluted	0.067	negative
SVC1232	undiluted	0.068	negative
SVC1231	undiluted	0.088	negative
VHS1016	undiluted	0.774	positive
VHS1015	undiluted	0.413	positive
VHS1014	undiluted	1.055	positive
VHS1034	undiluted	0.695	positive
Positive control		2.292	Valid

Bio-X Diagnostics
Site du complexe des Postes 49, rue Joseph Wauters BE-5580 Jemelle BELGIUM (Europe)
Tel: +32 (0)84.32.23.77 Fax: +32 (0)84.31.52.63 E-mail: info@biox.com http://www.biox.com

Conclusions

VHSV detectability between $10^{5,5}$ and $10^{6,75}$ / ml

No crossreactivity detected with other Rhabdoviruses.

The European Community Reference Laboratory for Fish Diseases
National Veterinary Institute
Technical University of Denmark
Hangovej 2
DK-8200 Arhus N
Denmark
December 18, 2009

ISOLATE	Geno-type	Anti-VHSV anti-body		Anti-IHNV antibody	
		Delta OD	Val	Delta OD	Val
DK-F1	I	1,293	72	-0,001	0
DK-Hededam	I	0,395	22	0,011	1
DK-3592B	Ia	0,696	39	0,041	2
DK-3971	Ia	0,961	53	-0,003	0
DK-3946	Ia	0,984	55	0,001	0
DK-5151	Ia	0,589	33	-0,002	0
DK-6137	Ia	0,789	44	-0,011	-1
DK-7974	Ia	1,059	59	0,006	0
DK-9695377	Ia	1,069	59	0,001	0
DK-200051	Ia	1,024	57	-0,002	0
DK-200149	Ia	1,198	67	0,002	0
FR-07-71	Ia	0,916	51	0,000	0
FR-23-75	Ia	1,097	61	-0,011	-1
FR-02-84	Ia	0,455	25	0,002	0
CZ-R5	Ia	0,761	42	-0,017	-1
CZ-2077	Ia	1,088	60	-0,008	0
DK-5927	Ia	1,104	61	-0,001	0
AU-8/95	Ia	0,742	41	0,001	0
CH-F1 262 BFH	Ia	0,467	26	-0,004	0
PL-202473	Ia	1,101	61	-0,006	0

ISOLATE	Geno-type	Anti-VHSV anti-body		Anti-IHNV antibody	
		Delta OD	Val	Delta OD	Val
DK-M Rhabdo	Ib	0,963	54	-0,005	0
1p8	Ib	0,850	47	-0,004	0
Ap40	Ib	0,814	45	-0,006	0
Control BioX		1,800	100	2,101	100
1p85	Ib	0,867	41	0,004	0
1p86	Ib	0,779	37	0,000	0
1p93	Ib	1,007	48	0,002	0
1p116	Ib	0,971	46	-0,001	0
1p20	Ib	0,896	42	0,002	0
1p121	Ib	0,998	47	-0,004	0
5p276	Ib	0,530	25	-0,004	0
SE-SVA-14	Ib	0,851	40	0,001	0
SE-SVA-1033	Ib	1,139	54	-0,002	0
UK-96-43	Ib	0,808	38	-0,005	0
4p37	Ib	0,285	13	0,000	0
FiA01a00 200198-1	Id	0,788	37	-0,002	0
FiP02b00 200240	Id	0,520	25	0,031	1
No-A136-68 EG46	Id	0,651	31	-0,024	-1
Ge-1.2	Ie	0,395	19	-0,003	0
TR-206239-1	Ie	0,890	42	0,020	1
1p49	II	0,990	47	-0,001	0
1p52	II	0,387	18	0,022	1
1p53	II	0,780	37	0,000	0
1p54	II	0,800	38	-0,002	0
2p51	III	0,950	45	-0,002	0
4p101	III	0,867	41	0,005	0
4p168	III	0,845	40	-0,013	-1
Control BioX		2,113	100	2,292	100
DK-4p51	III	1,019	49	0,001	0
UK-H17/5/93	III	0,954	46	-0,004	0
UK-860/94	III	1,389	66	-0,001	0
UK-H17/2/95	III	1,027	49	-0,005	0

Bio-X Diagnostics
Site du complexe des Postes 49, rue Joseph Wauters BE-5580 Jemelle BELGIUM (Europe)
Tel: +32 (0)84.32.23.77 Fax: +32 (0)84.31.52.63 E-mail: info@biox.com http://www.biox.com

ISOLATE	Geno-type	Anti-VHSV anti-body		Anti-IHNV antibody	
		Delta OD	Val	Delta OD	Val
F-L59x	III	0,983	47	0,000	0
GH30	III	0,569	27	-0,002	0
IR-F13.02.97	III	1,063	51	0,000	0
NO-2007-50-385	III	0,994	47	-0,001	0
USA-Makah	IVa	0,624	30	0,003	0
USA-KHV	IVa	0,672	32	-0,003	0
USA-Elliot Bay	IVa	0,626	30	0,000	0
Minter Creek, WA	IVa	0,903	43	-0,005	0
Tokul Creek, WA	IVa	0,988	47	-0,002	0
Port Angels, WA	IVa	0,849	41	-0,001	0
BC93	IVa	0,624	30	-0,001	0
CAN-3624	IVa	0,821	39	0,000	0
CAN-99-019	IVa	0,826	39	0,003	0
Quatsini, BC	IVa	0,919	44	-0,003	0
JP-Obama 25	IVa	0,570	27	-0,004	0
JF00Ehi1	IVa	0,557	27	-0,003	0
BRO1Ehi1	IVa	0,309	15	0,001	0
JF01Oit1	IVa	0,241	12	0,000	0
JSL02 Yaml	IVa	0,740	35	-0,005	0
Control BioX		2,095	100	2,395	100
PMO5Ehi1	IVa	0,496	25	-0,007	0
Lake St. Clair	IVb	0,715	36	0,002	0
Goby 1-5	IVb	0,775	40	-0,007	0
Lake Ontario, NY	IVb	0,693	35	0,003	0
Budd Lake, MI	IVb	0,805	41	-0,001	0
Skaneateles Lake, MI	IVb	0,820	42	-0,005	0
New Brunswick	IVb	0,940	48	0,002	0
1p40	Ib	0,848	43	-0,002	0
DK-5131	Ia	0,633	32	-0,004	0
DK-2835		0,976	50	-0,002	0
DK-5123		1,056	54	-0,004	0
Skaneateles Lake, 2 clone	IVb	0,712	36	0,004	0
Control BioX		1,961	100	2,265	100

Bio-X Diagnostics
Site du complexe des Postes 49, rue Joseph Wauters BE-5580 Jemelle BELGIUM (Europe)
Tel: +32 (0)84.32.23.77 Fax: +32 (0)84.31.52.63 E-mail: info@biox.com http://www.biox.com

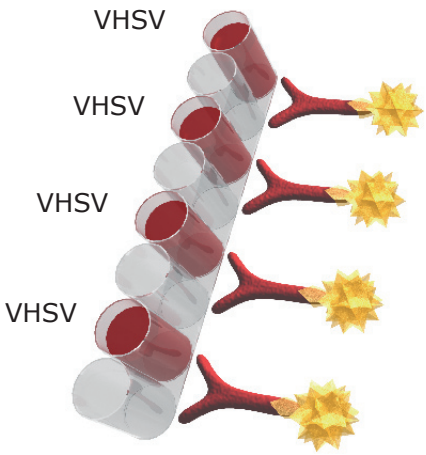
Conclusions

The Bio-X VHSV ELISA kit detects all the tested VHSV isolates with correct results.
The specificity is 100 % by testing all IHNV positive in the VHSV test.

Composition of the kit

BIO-X VHSV ELISA KIT : BIO K 272

	BIO K 272/1	BIO K 272/2
Microplates	1 (48 tests)	2 (96 tests)
Washing solution	1 X 100 ml (20 X)	1 X 100 ml (20 X)
Conjugate	1 X 12 ml (1 X)	1 X 25 ml (1 X)
Positive control	1 X 2 ml (1 X)	1 X 4 ml (1 X)
Single component TMB	1 X 12 ml (1 X)	1 X 25 ml (1 X)
Stopping solution	1 X 6 ml (1 X)	1 X 12 ml (1 X)



Stability : One year between +2°C and +8°C

Bio-X Diagnostics
Site du complexe des Postes 49, rue Joseph Wauters BE-5580 Jemelle BELGIUM (Europe)
Tel: +32 (0)84.32.23.77 Fax: +32 (0)84.31.52.63 E-mail: info@biox.com http://www.biox.com

Bio-X Diagnostics

VHSV ELISA KIT ANTIGENIC

DIRECT TEST

DIAGNOSIS TEST OF VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS (VHSV)

SANDWICH ELISA TEST

I - INTRODUCTION

Viral haemorrhagic septicaemia (VHS) is a rhabdovirus infection that is very common on the European continent. It causes great losses on European trout farms, where an estimated 20 to 30 thousand tons of trout are lost to this disease each year (de Kinkelin et al., 1985). Three distinct serotypes of this virus have been identified. The rainbow trout (*Oncorhynchus mykiss*) appears to be very sensitive to this virus. However, other Salmonidae, such as the brown trout (*Salmo trutta fario* L.) (de Kinkelin & Le Berre, 1977; Jorgensen, 1980), grayling (*Thymallus thymallus* L.) and even white fish may develop the disease. Salmonidae hybrids show variable degrees of sensitivity to the virus. The pike (*Esox lucius* L.) is also sensitive to VHS, primarily as fry (Meier & Jorgensen, 1973; Dorson et al., 1987), but the adult fish is also vulnerable (Lehmann et al., 1989). The pathogenic power of this virus is expressed only in water that is colder than 14° C. This explains why the disease strikes primarily in the winter.
The clinical signs of the disease are high mortality, especially during the young trout's first winter. The subjects exhibit melanosis and exophthalmia. The paleness of their gills reflects their anaemic condition. An autopsy will reveal the presence of numerous sites of haemorrhages in the viscera and muscle mass. It is practically impossible to distinguish VHS from infectious haematopoietic necrosis (IHN) - another Salmonidae viral infection likewise caused by a rhabdovirus - on the basis of clinical examination alone. A differential diagnosis must thus be performed in a laboratory. While neutralising antibodies have been identified (de Kinkelin et al., 1977), the clinical diagnosis is most often confirmed by isolating the virus in a cell culture.
The VHSV ELISA test confirms the virus's growth on a susceptible cell line.

II - PRINCIPLE OF THE TEST

The infected specimens are ground up in a mortar with the help of sand, then put in solution in an antibiotic-supplemented culture medium. The preparation is centrifuged and a 24-well cell culture plate is inoculated with a serial dilution of the supernatant. After 1 hour's incubation at optimal temperature culture medium is added to each well and the plate is incubated until a cytopathogenic effect is observed. At this point, the plate is frozen. It is ready to be tested by ELISA. The test uses 96-well microtitration plates sensitised by specific antibodies for the VHS virus. Rows A, C, E, G have been sensitised with these antibodies and rows B, D, F, H contain non specific antibodies. These control rows allow the differentiation between specific immunological reactions and non specific binding so as to eliminate false positives.
The supernatants are incubated on the microplate for 1 hour at 21°C +/- 3°C.
After this first incubation step, the plate is washed and incubated for 1 hour with the conjugate, a peroxidase labelled anti-VHSV specific monoclonal antibody. After this second incubation, the plate is washed again and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic.
If VHSV is present in the cell culture supernatant, the conjugate remains bound to the corresponding microwells and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of VHSV in the supernatant. The enzymatic reaction can be stopped by acidification and the resulting optical density at 450 nm recorded using a photometer. The signals recorded for the negative control microwells are subtracted from the corresponding positive microwells. A positive control antigen is provided with the kit so as to validate the test results.

III - COMPOSITION OF THE KIT

- **Microplates**: 96-well microtitration plates. Rows A, C, E, G are sensitised by anti-VHSV specific antibodies, while rows B, D, F, H are sensitised by the non specific antibodies.
- **Washing solution**: Bottle concentrated washing solution. The solution crystallises spontaneously when cold. If only part of the solution is to be used, bring the bottle to 21°C +/- 3°C until disappearance of all crystals. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water. Store the diluted solution between +2°C and +8°C.
- **Conjugate**: Vial of coloured conjugate. **This solution is ready to use.**
- **Positive Control:** The reagent is ready to use.
- **Single component TMB** Bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and +8°C protected from light. **This solution is ready to use.**
- **Stopping solution**: Bottle of the 1 *M* phosphoric acid stop solution.

	BIO K 272/1	BIO K 272/2
Microplates	1	2
Washing solution	1 X 100 ml (20 X)	1 X 100 ml (20 X)
Conjugate	1 X 12 ml (1 X)	1 X 25 ml (1 X)
Positive control	1 X 2 ml (1 X)	1 X 4 ml (1 X)
Single component TMB	1 X 12 ml (1 X)	1 X 25 ml (1 X)
Stopping solution	1 X 6 ml (1 X)	1 X 15 ml (1 X)

IV - ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

Distilled water, graduated cylinders, beakers, plastic tubes, tube rack, dispenser tips, reagent reservoir for multichannel pipettes, lid, adhesive for microplates, graduated automatic (mono- and multichannel) pipettes, microplate reader, and microplate washer and shaker (optional)

V - PRECAUTIONS FOR USE

- This test may be used for "in vitro" diagnosis only. It is strictly for veterinary use.
- The reagents must be kept between +2°C and +8°C. The reagents cannot be guaranteed if the shelf-life dates have expired or if they have not been kept under the conditions described in this insert.
- The concentrated wash solution may be stored at room temperature. Once diluted, this solution remains stable for six weeks if kept between +2°C and +8°C.
- Unused strips must be stored immediately in the aluminium envelope, taking care to keep the desiccant dry and the envelope's seal airtight. If these precautions are taken, the strips' activity can be conserved up to the kit's shelf-life date.
- Do not use reagents from other kits.
- The quality of the water used to prepare the various solutions is of the utmost importance. Do not use water that may contain oxidants (e.g., sodium hypochlorite) or heavy metal salts, as these substances can react with the chromogen.
- Discard all solutions contaminated with bacteria or fungi.
- The stop solution contains 1 M phosphoric acid. Handle it carefully.
- All materials and disposable equipment that come in contact with the samples must be considered potentially infectious and be disposed of in compliance with the legislation in force in the country.
- To guarantee the reliability of the results, one must follow the protocol to the letter. Special care must be taken in observing the incubation times and temperatures, as well as measuring the volumes and dilutions accurately.

VI – PROCEDURE

For countries belonging to the European Communities, sampling plans and diagnostic methods for the detection and confirmation of viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) must be applied (2001/183/EC) – Commission decision of 22 February 2001.

1. Extracting the virus

1.1. Preparing the specimens

Take from moribund fish or fresh corpses approximately 1-gram fragments of spleen, kidney and brain tissue. Mix these fragments with oven-sterilised sand and grind the mixture in a mortar. After complete homogenisation is achieved add 2 ml of culture medium containing 2% foetal calf serum and antibiotics

(inoculation medium). For example, one may use a mixture of 200 IU of penicillin, 200 µg of streptomycin and 200 µg of kanamycin per ml of culture medium. This mixture avoids the problems encountered when cell cultures are inoculated with heavily-contaminated specimens. For small fish, the entire corpse may be homogenised in the mortar, ideally after the intestines have been resected.

1.2. Centrifugation of specimens
The homogenised preparation is centrifuged at between 2,000 and 4,000 g at 4° C for 15 minutes. The supernatant is collected for the subsequent steps.

1.3. Dilution of specimens
1:10, 1:100 and 1:1,000 dilutions of the supernatant are made using the inoculation medium.

2. Isolating the virus

2.1. Cell line selection
The FHM, EPC, BF.2 and RTG2 cell lines are susceptible to VHS virus. These cells may be grown in Eagle's modified MEM or with better results in Glasgow's MEM supplemented with 10% foetal calf serum, 10% phosphate tryptose and a mixture of antibiotics at the standard concentration. If a CO2 incubator is not available, the medium may be buffered at pH 7.4 with 0.16 M Tris-HCl. The optimal temperature for growth is 30° C for the FHM and EPC cells, 25° C for the BF.2 cells and 21° C for the RTG2 cells (de Kinkelin et al., 1986).

2.2. Preparation of the cellular substrate
The cells are kept in a Roux flask at their optimal growth temperature. One to two days before use the cells are treated with trypsin to separate them from their backing, then seeded on a 24-well plate.
As susceptibility to the virus depends on the cells' age, it is advisable to use them 24-48 hours after their transfer to the plate. To guarantee the quality of the diagnosis, the cell layer must be in perfect condition at the time of inoculation with the specimens.

2.3. Inoculation
The culture medium is eliminated by turning the plate upside down over a receptacle. Use a sharp movement so as to avoid adsorption of the culture medium on the outer surface of the well. In carrying out this step, hold the plate at a reasonable distance from the receptacle to avoid all risks of contamination from splashes. After emptying the plate, quickly deposit the different dilutions of specimens, for the cell layer must be kept moist at all times. The specimens must be deposited in the wells very delicately so as not to damage the cell layer. If automatic microtip pipettes or Pasteur pipettes are used, place the tip of the pipette against the side wall of the well and release the sample material slowly. 200 µl aliquots of the different dilutions are placed in each well. Incubate the plate at 15° C for 1 hour.

2.4. Addition of inoculation medium
At the end of the viral adsorption period add gently to each well 1 ml of the 2% foetal calf serum culture medium (inoculation medium).

2.5. Incubating the plate
The plate is kept in an incubator (under 5% CO2) at the optimum temperature for viral growth (15° C). It is inspected daily until a cytopathogenic effect is observed. This consists of the development of dense, spherical cells, the destruction of which results in the formation of plaques.

2.6. Freezing the plate
The plate is frozen in order to release the virus from cells

3. Identifying the virus

3.1-Bring all the reagents to 21°C +/- 3°C hour before use. Thaw the culture plate that was prepared from the samples.

3.2-Dilute the concentrated washing solution 20 fold in distilled water. Be sure that all crystals have disappeared before dilution.
Keep this solution between +2°C and +8°C when not used.

3.3-Add 100-µl aliquots of the supernatants to the wells as follows: sample 1 in wells A1 and B1, sample 2 in wells C1 and D1, etc. Proceed in the same manner for the positive reference (ex.: G1 and H1).
3.4-Incubate the plate at 21°C +/- 3°C for 1 hour. Cover with a lid.
3.5-Rinse the plate with the washing solution, prepared as instructed in step 3.2, as follows: Empty the microplate of its contents by flipping it over sharply over a vessel containing sodium hypochlorite. Tap the microplate upside down against a piece of clean absorbent paper to remove all the liquid. Fill all the used wells with the washing solution using a spray bottle or by plunging the plate in a vessel of the right dimensions, then empty the wells once more by turning the plate over above a sink with sodium hypochlorite. Repeat the entire operation two more times, taking care to avoid the formation of bubbles in the microwells. After the plate has been washed three times proceed to the next step.
3.6-Distribute the conjugate solution at the rate of 100 µl per well. Incubate at 21°C +/- 3°C for 1 hour. Cover the plate with a lid.
3.7-Wash the plate as described in Step 3.5.
3.8-Add 100 µl of the chromogen solution to each well on the plate. The chromogen solution must be absolutely colourless when it is pipetted into the wells. If a blue colour is visible, this means that the solution in the pipette has been contaminated. Incubate at 21°C +/- 3°C and away from light for 10 minutes. Do not cover. This time interval is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
3.9-Add 50 µl of stop solution to each well.
3.10-Read the optical densities by means of a microplate spectrophotometer with a 450 nm filter. The results must be read as quickly as possible after the stop solution has been applied, for in the case of a strong signal the chromogen can crystallise and lead to incorrect measurements

VII – INTERPRETING THE RESULTS

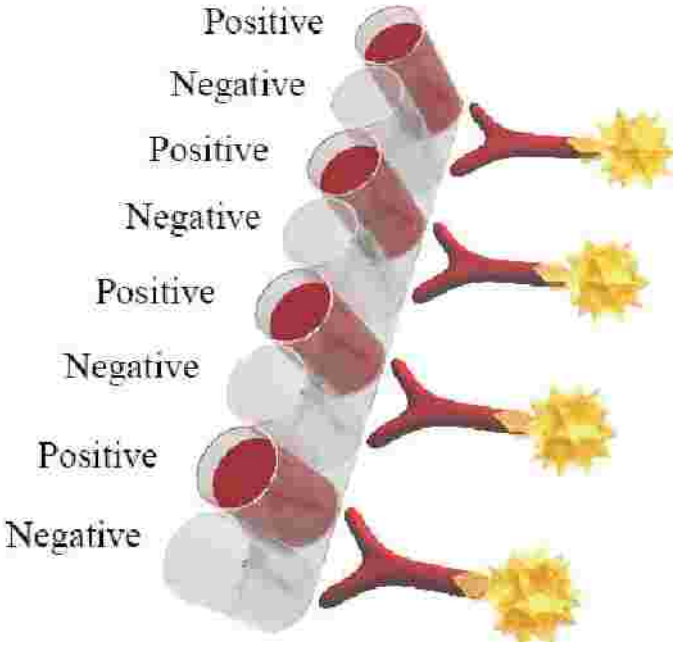
Calculate the net optical density of each sample by subtracting from the reading for each sample well the optical density of the corresponding negative control.
Proceed in the same way for the positive control antigen.
The test is validated only if the positive control antigen yields a difference in the optical density at 10 minutes that is greater than the value given on the QC data sheet.

Divide the signal read for each sample well by the corresponding positive control signal and multiply this result by 100 to express it as a percentage. Using the first table in the quality control procedure, determine each sample's status (positive, negative).

$$Val = \frac{\text{Delta DO spl} * 100}{\text{Delta DO pos}}$$

VIII– ORDERING INFORMATION

VHSV ELISA KIT :	1 X 48 tests	BIO K 272/1
	2 X 48 tests	BIO K 272/2



IX – REFERENCES

Dorson M., de Kinkelin P., Torchy C. and Monge D. (1987) Sensibilité du brochet (Esox lucius) à différents virus de salmonidés (NPI, SHV, NHI) et au rhabdovirus de la perche. Bull. Fr. Pêche Piscic., 307, 91-101.

Jorgensen P.E.V. (1980)
Egtved virus : the susceptibility of brown trout and rainbow trout to eight virus isolates and the significance of the findings for the VHSV control. Third session of the Cooperative Program of Research on Aquaculture (COPRAQ). Fish disease, Springer-Verlag Berlin Heidelberg New York, Ahne editor.

de Kinkelin P. and Le Berre M. (1977) Isolement d'un rhabdovirus pathogène de la truite fario (Salmo trutta). C.R. Acad. Sc. Paris, 284, série D, 101-104.

de Kinkelin P., Gérard J-P., Dorson M. and Le Berre M. (1977) Viral haemorrhagic septicaemia: demonstration of a protective immune response following natural infection. Fish Health News, 6, 3-4.

de Kinkelin P., Michel Ch. and Ghittino P. (1986) Précis de pathologie des poissons. Office International des Epizooties et Institut National de la Recherche Agronomique Editeurs, Paris, 348 pp.

Lehmann J., Mock D., Stürenberg F.J. and Ahne W. (1989) VHSV-epizootics in adult pike (*Esox lucius* L). Bull. Eur. Ass. Fish Pathol. 9 (3), 61.

Meier W. and Jorgensen P.E.V. (1973) Isolation of VHS virus from pike fry (*Esox lucius*) with hemorrhagic symptoms.

Third session of the EIFAC FAO/OIE Cooperative Program of Research on Aquaculture (COPRAQ) Fish disease. Munich (Congres 379).

Commission decision of 22 February 2001 laying down the sampling plans and diagnostic methods for the detection and confirmation of certain fish diseases and repealing Decision 92/532/EEC (2001/183/EC)