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(54) **Titre : AGENT AVEC DES PROPRIETES ANTIVIRALES POUR LA PREVENTION OU LE TRAITEMENT D'INDIVIDUS EXPOSES  
A UN VIRUS DE LA FAMILLE DES BIRNAVIRUS**

(54) **Title: AGENT WITH ANTIVIRAL PROPERTIES FOR PREVENTING OR TREATING INDIVIDUALS EXPOSED TO A VIRUS OF  
THE BIRNAVIRIDAE FAMILY**

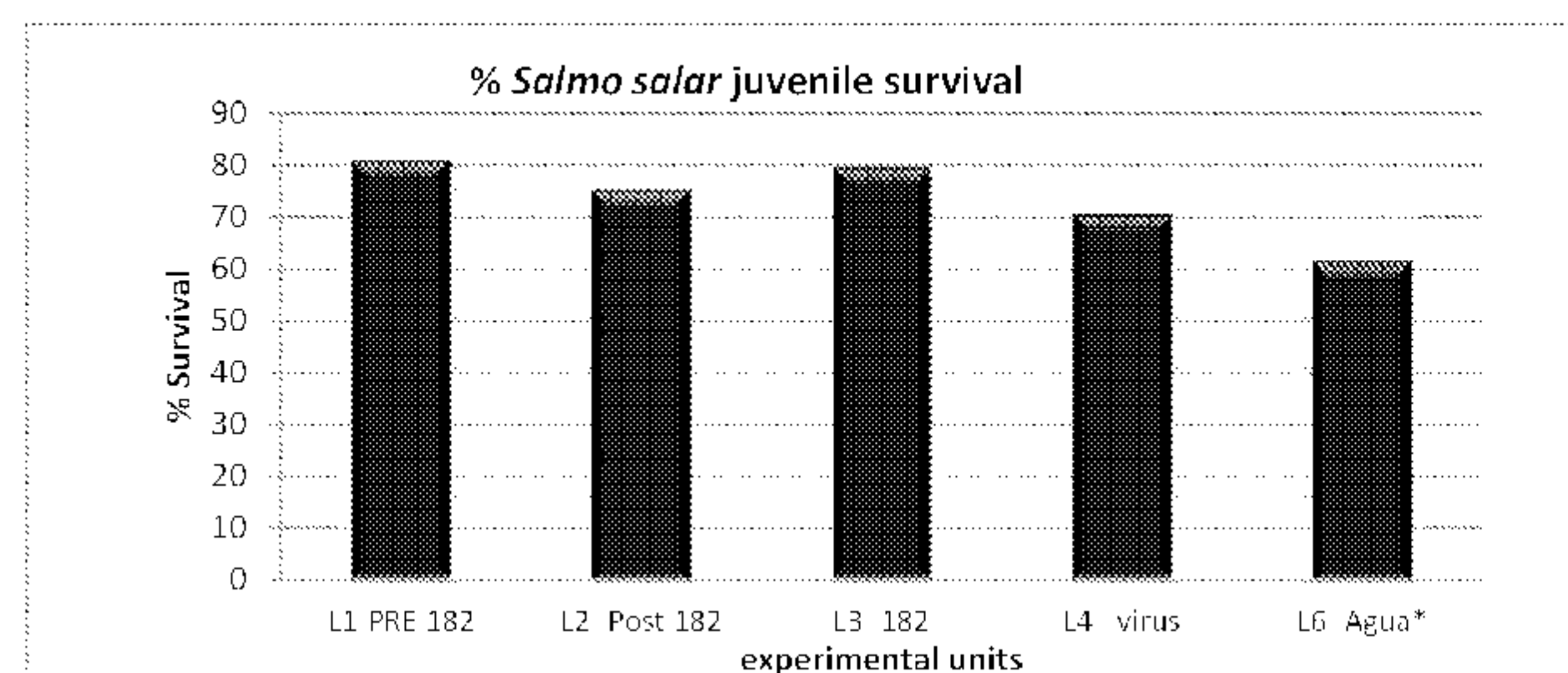


Figure 9

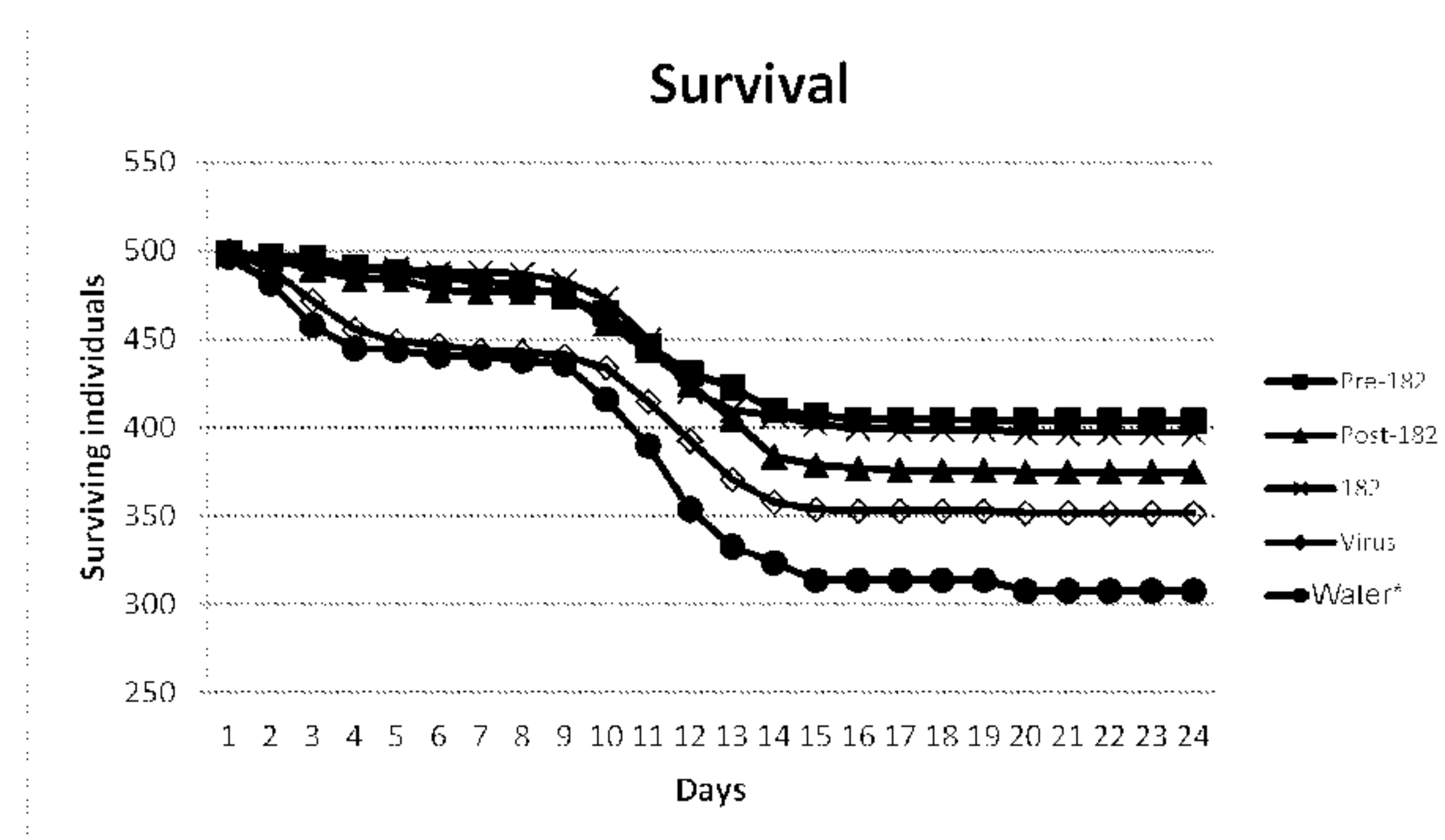


Figure 10

(57) **Abrégé/Abstract:**

The present invention relates to molecules with antiviral properties to prevent the spread of infection in animal or fish by a virus of the Birnaviridae family, specifically the infectious pancreatic necrosis virus (IPNV). More particularly, the invention pertains to

**(57) Abrégé(suite)/Abstract(continued):**

peptides that are effective in decreasing viral infection by reducing or impeding the production of viral particles, and can therefore prevent the potential mortality resulting from virus infection and is of particular use in the prophylaxis or prevention of infections by viruses of the Birnaviridae family. In a particular embodiment, we consider the use of peptides applied to the tanks where the fish are grown or as part of a feed composition.

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[Continued on next page]

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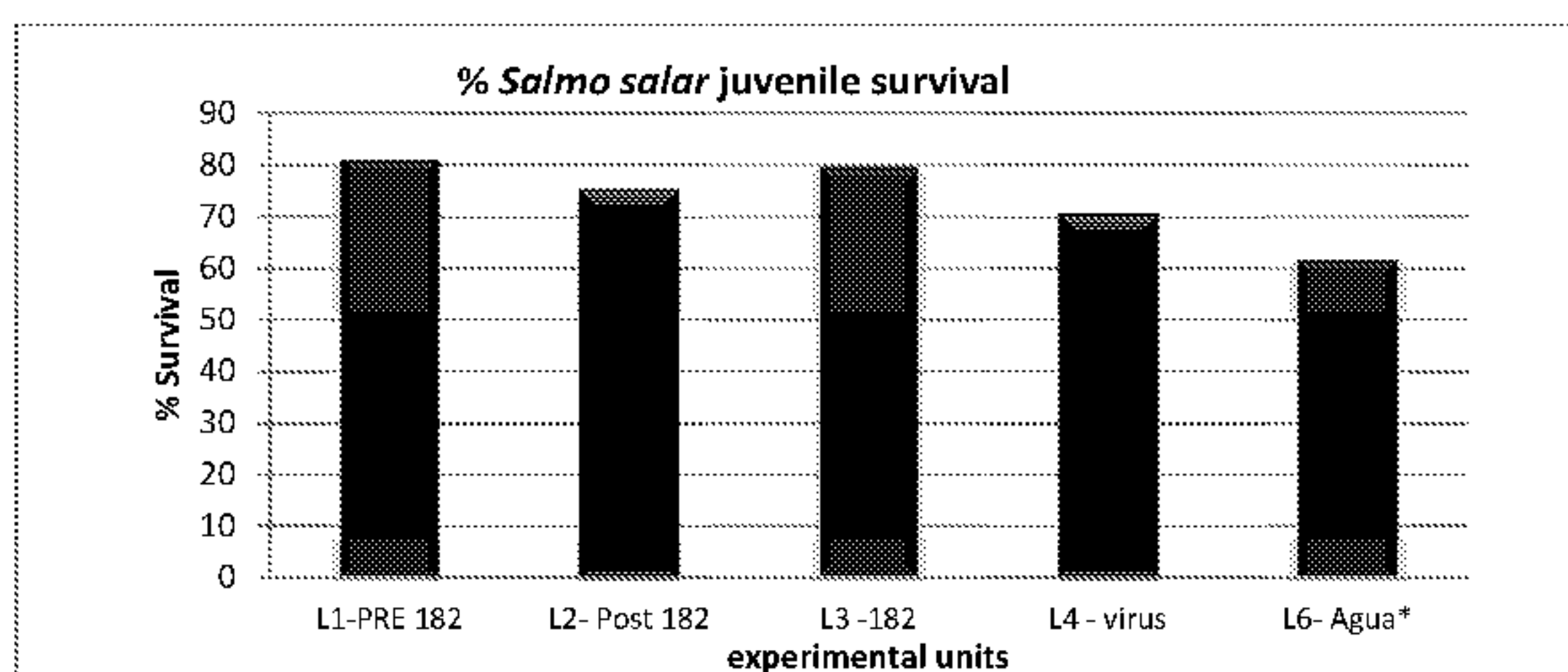


Figure 9

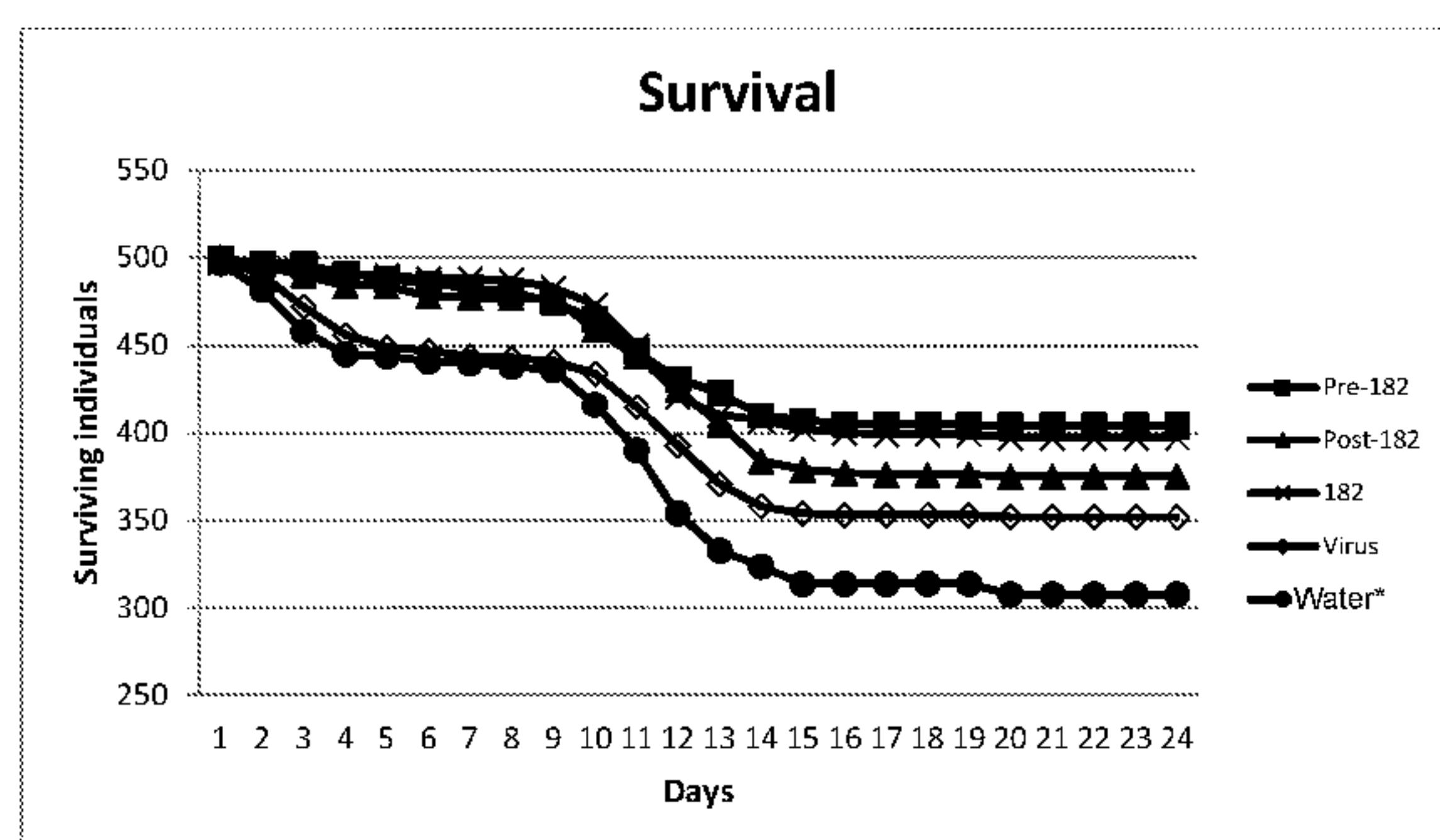


Figure 10

(57) **Abstract:** The present invention relates to molecules with antiviral properties to prevent the spread of infection in animal or fish by a virus of the *Birnaviridae* family, specifically the infectious pancreatic necrosis virus (IPNV). More particularly, the invention pertains to peptides that are effective in decreasing viral infection by reducing or impeding the production of viral particles, and can therefore prevent the potential mortality resulting from virus infection and is of particular use in the prophylaxis or prevention of infections by viruses of the *Birnaviridae* family. In a particular embodiment, we consider the use of peptides applied to the tanks where the fish are grown or as part of a feed composition.

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- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
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## AGENT WITH ANTIVIRAL PROPERTIES FOR PREVENTING OR TREATING INDIVIDUALS EXPOSED TO A VIRUS OF THE BIRNAVIRIDAE FAMILY

### TECHNICAL FIELD

The present invention relates to molecules that can be used as a prophylactic agent, therapeutic agent, a prophylactic food supplement with therapeutic potential, or an agent with antiviral properties which prevent infections or treat animals or fish that have been exposed to or are infected with a virus of the *Birnaviridae* family. Specifically, the invention pertains to peptides that are effective in decreasing viral infection by reducing or impeding the production of viral particles, and can therefore prevent the potential mortalities associated with virus infection.

### BACKGROUND AND PRIOR ART

*Birnaviruses* corresponds to a group of viruses possessing two segments of double-stranded RNA and belongs in group III according to the general classification of viruses. This group contain eight families, all of which have icosahedral symmetry, with a diameter of 60 nm and are "unwrapped". Present inside the virions are 5 proteins and between 3 and 4 peptides.

The *Birnaviridae* family consists of the *Aquabirnavirus*, *Avibirnavirus* and *Entomobirnavirus* genera. The *Aquabirnavirus* genus consists of birnavirus that infect fish, molluscs, crustaceans and rotifers. Species of the *Aquabirnavirus* genus are the causative agents of infectious pancreatic necrotic virus (IPNV), as well as the yellow tail ascites virus (YTAV), marine birnavirus (MABV). The *Blosnavirus* family includes the blotched snakehead virus (BSNV); the *Avibirnavirus* is a genus of viruses that affect specifically poultry, and corresponds to the cause of the Infectious Bursal Disease, which has great impact in poultry industry. In particular, the virus is Infectious Bursal Disease virus (IBDV); and finally, the *Drosophila X virus* is part of the *Entomobirnavirus* family and infects *Drosophila melanogaster* (fruit fly).

In the present invention, the infectious pancreatic necrosis virus (IPNV) is used as a prototype birnavirus to generate a generic alternative prophylactic that can be used against the whole *Birnaviridae* family of viruses. Therefore, the description provided in here below directed specifically to IPNV should be understood as an example and not to be used to limit the scope of the invention.

In a particular case, without intending to limit the scope of the present invention to a particular virus species, described below is one of many instances where a *Birnaviridae* family virus represents a major problem for a particular industry. This description should only be interpreted as an exemplification of one of the problem cases, bearing in mind that there are other members of the *Birnaviridae* family affecting other industries and these industries could also benefit from the present invention.

Chile is the second largest global competitor in salmon farming. However, the industry is subject to production highs and lows, which usually are caused by pathogens. Agents that have generated the greatest losses to salmon aquaculture are IPNV, ISA and the bacterium *P. salmonis* to name the most important. In the case of pancreatic necrosis virus (IPNV) statistics reveal losses of the order \$200 million per year.

This pathogenic agent is spread throughout all salmon producing countries and has a particular characteristic: it is latent in bottom sediments infecting different mollusks and native fish surrounding salmon farms without causing disease and can be isolated from fish farms in lakes. In addition, the largest outbreaks of disease occur during the early stages of development, i.e. first feeding stage and in the transfer of the fish smolt to the ocean producing outbreaks with up to 70% mortality.

To date there are several therapeutic tools against this infection with the greatest benefits coming from vaccines with antigenic action against the IPNV structural protein; however this type of protection can only be used in medium or large size fish.

For these reasons the generation of a new therapeutic alternative is needed to control the virus in its early stages and which can be used in the early stages of fish development and should be a complement to the tools developed up to now.

### **Infectious Pancreatic Necrosis Virus**

The aquatic *Birnavirus* are those with the greatest range of infection, infecting numerous fish species, among which are the Salmonids. The prototype of the aquatic *Birnaviridae* family is the Infectious Pancreatic Necrosis Virus (IPNV), considering members of this group, all are bisegmented double-stranded RNA viruses (dsRNA) able to cause clinical infection in one of the respective species. The first pathogenic birnavirus was isolated from a fish found in a brook trout (*Salvelinus fontinalis*) in the National Fish Hatchery, Leetown, West Virginia, USA. The agent was obtained during an epizootic of trout fingerlings suffering pancreatic necrotic infection (IPN). This necrotic pancreatic virus (IPNV) was deposited in the American Type

Culture Center (ATCC) as ATCC VR299 IPNV and has been detected for many years in various locations in North America associated with high mortality rates in juvenile trout.

Most aquatic birnaviruses are antigenically related, representing a major serogroup (A) with 10 serotypes and only a few birnavirus not antigenically related forming a second serogroup (B). Most of the IPNV isolated in the USA belong to serotype A1 (West Buxton); the Canadian isolates (C1, C2, C3, Jasper) to serotypes A6-A9 and the European isolates (Sp, Ab, I and Te) to serotypes A2-A5 and serotype A10. Serotypes A1, A2 and A3 have been detected in Asia..

### **IPNV genomic organization**

The aquatic birnaviruses have similarities in morphology and biochemical and biophysical properties. IPNV virions present the typical characteristics of the *Birnaviridae* family. The IPNV genome consists of two segments of double stranded RNA (dsRNA) of 3.1 kb (segment A) and 2.9 kb (segment B) with non-coding regions (UTR) at the 5' and 3'.

Segment A contains two partially overlapping open reading frames (ORFs). The first encodes a non-structural protein VP5 (145 aa, 17 kDa) which is a cytolytic membrane protein that while dispensable for virus replication in cell culture, is important in pathogenesis *in vivo* as it is involved in the release and dispersion of the viral progeny. It has been proposed that this protein inhibits apoptosis in the early stages of infection. The second ORF encodes a polyprotein of 107 kDa (972 aa) which is proteolytically autoprocessed leading to the pVP2 protein (508 aa, 54 kDa), VP4 (225 aa, 25 kDa) and VP3 (237 aa, 28 kDa) in a co-translational process mediated by VP4 itself. pVP2 is the precursor form of the capsid structural protein, VP2. The 74 carboxy terminal residues of pVP2 are processed to yield the mature form VP2 (442 aa, 48kDa). In any case, the maturation process requires assembly of the viral capsid and the small polypeptides generated are retained in the viral particles. Such peptides have the ability to disrupt membranes. As occurs in morphogenetic processes in other viral systems, this proteolytic maturation process could confer irreversibility to the capsid assembly.

### **Viral Proteins**

As mentioned above, IPNV needs to process its own proteins to be infective. Besides possessing five major proteins including VP2, VP3 and VP4 it generates a number of peptides resulting from the processing of the polyprotein.

Studies of the morphology of IPNV found two types of viral particles, A particles or provirion, which is the first particle detectable post infection simultaneously with the double-stranded

RNA, suggesting that the virus assembly occurs as soon as the double-stranded RNA is replicated. It has also been determined that the particle is not found fully assembled: it follows that it corresponds to an intermediary in morphogenesis. After maturation of the provirions, infective B particles are produced (virions; 3-4 hours post- infection), where mature and completely folded proteins were found. Thus it is concluded that the maturation of IPNV A particles not only indicates the acquisition of infectivity, but also the reduction in the diameter of the particles. This is demonstrated by densitometry analysis of viral proteins made by the two types of particles (A and B), wherein it is seen that the type A particles have mostly VP2 capsid protein in the non- mature pVP2 form, and that type B particles have only VP2 and not the intermediary pVP2 .

For the pVP2 maturation process three cleavage sites were identified. Two of these cleavage sites (486-487, 495-496) were proposed as a target of the VP4 viral protease. Of these two sites, the primary cleavage site at the junction pVP2-VP4, is defined as a [S / T] XAA motif. This consensus sequence shows some similarity to the SKAW sequence found in the 442-443 region, suggesting that the VP4 could be involved in the cleavage to generate mature VP2.

Furthermore, recombinant expression of truncated VP2 was detected in CHSE-214 cells at nanogram concentrations of monoclonal antibody CE4 by ELISA and Immunoblotting. This observation suggests that both the folding and glycosylation of truncated VP2 could be extremely similar to the native viral protein.

It has been shown that the majority of neutralizing monoclonal antibodies react with epitopes within the VP2 protein, it has also been suggested for neutralizing epitopes for VP3. Three epitopes have also been found, two variable and one conserved, located in the central third of VP2.

### **Apoptosis in CHSE-214 cells infected with IPNV**

Apoptosis is a process of carefully regulated cell death that can be triggered by a variety of stimuli, one of these being viral infection. Some features of viral infection are recognized by cells as harmful, promoting a defensive response that ends with cell death. Induction of apoptosis in most cases is a challenge for successful viral replication. But sometimes appropriate manipulation of the apoptotic response results in a cell death mechanism which increases the development of the virus. IPNV, like many other viruses, induces apoptosis in cultured cells. This response is seen during the early stages of IPNV multiplication involving severe morphological damage to the infected cells for example, such as the blebbing or

bubbling of plasmatic membrane. The expression of this cell death program can be interpreted as a defense mechanism of the cell against IPNV multiplication. However, most infected cells lack an apoptotic response, at least half of the infected cells do not express apoptotic signals at any time during the viral replication.

It has been shown that the Annexin V assay is sensitive to the earliest morphological changes produced in the cell, and can be used as an early marker of apoptosis, including apoptosis detected before the activation of the caspases.

### **Susceptible hosts and geographic distribution**

As mentioned above, aquatic birnaviruses are viruses that have a great range of action, with representatives infecting numerous fish species, where salmonid fish are highlighted, for example rainbow trout (*Oncorhynchus mykiss*), river trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), lake trout (*Salvelinus namaycush*), Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), and numerous invertebrates and marine fish such as eels (*Anguilla anguilla*, *Anguilla japonica*) striped fish, tilapia (*Tilapia mossambica*), marine molluscs and crustaceans of European and Japanese coasts. Consequently, according to the regions or habitats of species referred to, IPNV is considered to be endemic in many parts of America, Europe and Asia, not being exclusive to Chile.

### **Methods of transmission**

In particular, IPNV is transmitted via feces, urine and sexual secretions of infected fish. For this reason it can be transmitted vertically via eggs. Studies of factors affecting the transmission and outbreaks of IPN indicate that iodophor used as disinfectant during the artificial fertilization process does not completely eradicate IPNV infectivity. The virus is also transmitted horizontally, surviving fish from IPNV outbreaks are transformed into vectors and can carry the virus throughout their lifetimes. IPNV can also be transmitted through the feces of fish-eating birds.

### **IPNV inhibitory peptides**

Previous studies have demonstrated the effectiveness of the use of synthetic peptides in the control of certain viral diseases. In Chile, the virus of infectious pancreatic necrosis virus (IPNV) has become a fastidious pathogen severely affecting the salmon industry every year. It is therefore very urgent to find an effective method for its control. The present invention is based on the use of chemically synthesized peptides for the purpose of inhibiting the assembly and /

or attenuating the infectivity of IPNV in an *in vitro* model as well as in large scale trials.

For the completion of this document, a search for prior art was conducted during which, publication of related patents were found. These are briefly described below.

The document WO1994004565 discloses synthetic polypeptides with antigenic properties useful in treating IPNV infections in fish, however, unlike the present invention it focuses on generating an immune response in fish, while in the present invention there is provided a prophylactic formulation which seeks to prevent transmission when an animal is exposed to the presence of one of the viruses under consideration, in particular IPNV.

US5165925 discloses a vaccine against VR-299 strains and SP of IPNV, where the vaccine is a polypeptide segment obtained from a segment of the virus. In particular it is mentioned that this segment includes at least VP2.

The documents US5780448 and US6180614 describe DNA vaccines where the vaccine consists of a DNA construct that encodes at least one peptide of a pathogen that attacks aquaculture species. Specific examples which comprise sequences encoding VP2, VP3, which could be combined are described.

US6010705 discloses a vaccine based on a live attenuated virus against the microorganism *Edwardsiella ictaluri*. It additionally discloses that the vaccine may further comprise a coding sequence of VP2 of IPNV (claim 5).

US20040047881 describes recombinant microorganisms which express portions of different pathogens. In particular one example is described in which the microorganism expresses part of segment A of IPNV. Finally, the microorganisms that express pathogen polypeptides are used to produce food for aquatic animals.

US20070286871 discloses a composition for preventing viral infection, where the composition generally comprises of an antigen derived from a virus and the antigen is from IPNV.

US20070248623 describes constructs encoding various virus antigens, among which is mentioned IPNV and a DNA vaccine comprising IPNV sequences.

US20100316663 describes a vaccine which corresponds to a fusion protein between the translocation domain of *Pseudomonas aeruginosa* exotoxin A and an antigenic protein of IPNV.

US20120040010 describes a vaccine formulation comprising excipients allowing the

composition to adhere to the mucous membranes of animals, within which fish are reported. It is mentioned that the vaccine can be against IPNV.

US8168201 describes a vaccine comprising a truncated VP2 antigen of IPNV and also considers the nucleic acid sequence encoding the truncated polypeptide.

EP1975238 discloses a vaccine for aquatic species. The specification mentions that the vaccine can be against IPNV, where the antigens may be from VP1, VP2, VP3.

WO2002038770 describes vaccines based on polypeptides VP2 and / or VP3 of IPNV.

WO2003015714 describes a composition which blocks viral replication (viral budding), wherein the active agent corresponds to a molecule which corresponds to the fusion of a transporter and a peptide that is part of a viral structure. It was mentioned that said viral structure may be a peptide of at least 6 amino acids, and also indicates that it can, in a particular instance, come from the IPNV VP2 protein. Nevertheless, the indication of the fusion peptide has a completely different functionality than the present invention, wherein a first peptide of the ones disclosed in WO2003015714 helps or improves the entry of a second peptide in the cell.

WO2003013597 discloses a vaccine consisting of sub-units of the viral capsid (VP2, VP3) that form an empty capsid.

WO1999050419 discloses a method for producing a vaccine against IPNV. The vaccine is an attenuated virus.

WO2008140610 describes a vaccine based on the VP2 viral capsid protein. The method of administration is by injection, or feeding of recombinant yeast strains that express the active agent (VP2) of the vaccine.

WO2011138489 describes the use of casein hydrolysates as antiviral agents.

The document US8168201, in particular mentions the use of a truncated antigen from VP2.

Furthermore, document WO1994004565 discloses synthetic peptides, where key positions are identified within peptides (R1, R2, R3) which can be changed to different amino acids options.

There are many patent documents which disclose some uses of nucleotide or amino acid sequences of the VP2 protein from IPNV, and although the present invention pertains to peptides derived from the sequence of VP2 of IPNV, it also identifies essential positions within the peptides developed.

The difference of the present invention with respect to the prior art is that the present invention is used as an antiviral agent, which prevents the infection of fish, where the molecules of the present invention can be used as a prophylactic agent, a therapeutic agent, a prophylactic food supplement with therapeutic potential, which have antiviral properties, where its application, e.g. through a bath, or through a special formulation or through a food matrix provided to animals or fish to be treated, either preventively or as an ongoing treatment of disease, as a dietary supplement or as a bath, where the peptides may be provided to the affected fish or animals during certain periods. Furthermore, the compositions of the present invention impedes the increase in viral load in chronically infected fish, or fish that are normally exposed to viral particles.

## BRIEF DESCRIPTION OF FIGURES

Figure 1: Inhibition of infective units by p20. Semi-quantitative immune detection of IPNV in *de novo* infected CHSE-214 cells shows the inhibitory action of p20. A. Positive control. Cells infected in the absence of peptide. B. Cells infected in the presence of 10 uM of p20. C. Negative control. Cells infected in the presence of peptide K-1. D. Focal Fluorescence Units on chart (left to right, respectively) shows mean and SD values.

Figure 2: Antiviral Activity of peptide p20 A. CHSE-214 cells permanently infected with IPNV, the number of copies of mRNA from VP2 of IPNV was measured with RT-qPCR from supernatant of cells. B. Calibration curve of number of copies of Topo-VP2 IPNV for qPCR.

Figure 3: Relative quantification of VP2 relative to elongation factor ELF. Treatment with the peptide before infection with IPNV.

Figure 4: Relative quantification of VP2 relative to elongation factor ELF. Treatment with the peptide after infection with IPNV.

Figure 5: Relative quantification of VP2 relative to elongation factor ELF. Treatment with peptide with alanine variations before infection with IPNV.

Figure 6: Relative quantification of VP2 relative to elongation factor ELF. Treatment with peptide 182 with alanine variations after infection with IPNV.

Figure 7: Change in viral titre of IPNV in CHSE-214 cells determined by focal fluorescence in a treatment with the peptide 182 before infection with IPNV.

Figure 8: Change in viral titre of IPNV in CHSE-214 cells determined by focal fluorescence in a treatment with peptide 182 after infection with IPNV.

Figure 9: Percentage cumulative survival after experimental trials to test effectiveness of the peptide 182 *in vivo*.

Figure 10: Trend of mortality shown in trials. No mortality present after day 24 until day 56.

Figure 11: Control versus treatment with peptide; All Results.

Figure 12: despite the heterogeneity of viral load per fish, the presence of peptides in the food tends to lower the overall viral load, reflected in the tendency to increase the relative values of Ct in time in clear difference with the control formulation without peptide (f-5) where the trend is to maintain or even increase the average viral load (higher Ct values).

## SUMMARY OF THE INVENTION

The present invention relates to molecules with antiviral properties to prevent the spread of infection in animal or fish by a virus of the *Birnaviridae* family, specifically the infectious pancreatic necrosis virus (IPNV). More particularly, the invention pertains to peptides that are effective in decreasing viral infection by reducing or impeding the production of viral particles, and can therefore prevent the potential mortality resulting from virus infection and is of particular use in the prophylaxis or prevention of infections by viruses of the *Birnaviridae* family. In a particular embodiment, we consider the use of peptides applied to the tanks where the fish are grown or as part of a feed composition.

## DETAILED DESCRIPTION OF THE INVENTION

As mentioned, the present invention pertains to synthetic peptides or fragments thereof, specially designed for the prophylactic control of viral diseases of the *Birnaviridae* family, more particularly the infectious pancreatic necrosis virus IPNV which affects many species of fish.

The present invention, in a first aspect, corresponds to synthetic peptides or fragments thereof having the property of reducing the rate of infection in animals exposed to a particular virus.

In one aspect, the specially processed synthetic peptides were developed based on a

sequence encoding pVP2 of the IPNV capsid.

More particularly, the peptides considered herein correspond to peptides with an identity of at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 98%, at least 99% compared to the amino acid sequences SEQ ID NO:1 (p182), SEQ ID NO:2 (p20), or fragments thereof.

In another embodiment, the peptides considered herein correspond to peptides with an identity of at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 98 %, at least 99%, with respect to the nucleotide sequences SEQ ID NO:3 (p182), SEQ ID NO:4 (p20), or fragments thereof.

In a second aspect, the present invention comprises a prophylactic agent; therapeutic agent; prophylactic food supplement with therapeutic potential, wherein said prophylactic agent; therapeutic agent; prophylactic food supplement with therapeutic potential corresponds to at least one or both peptides described above or fragments thereof, that is, comprises at least one, two or three peptides having an identity of at least 80%, at least 85%, at least 90 %, at least 92 %, at least 95 %, at least 98 %, at least 99 % compared to the amino acid sequences SEQ ID NO:1, SEQ ID NO:2, or fragments thereof, or at least one, two, or three peptides having an identity of at least 80%, at least 85 %, at least 90 %, at least 92 %, at least 95 %, to least 98%, at least 99 %, with respect to the nucleotide sequences SEQ ID NO:3, SEQ ID NO:4, or fragments thereof; and one or more pharmaceutically acceptable excipients.

In a third aspect of the invention, an application of the prophylactic formulation of the invention to animals at risk of potential contact with the source of infection of a virus of the *Birnaviridae* family is considered. In a particular embodiment, the animal at risk of exposure to a virus of the *Birnaviridae* family is a fish. In a more specific embodiment, the virus of the *Birnaviridae* family is IPNV.

In yet another aspect, particularly when considering the embodiment of the invention as a prophylactic dietary supplement with therapeutic potential, the integration of the peptides of the invention in a food matrix in such a way that they can be administered or provided to growing animals is considered. According to this aspect of the invention, the peptides described above are administered to farmed fish through a composite food. Since it is not possible to measure accurately the quantities of peptides that are actually consumed by each fish, ranges of amount of peptides administered in a composite food have been set. Thus, for example, in the particular embodiment of application through food, the peptides of the invention

according to the amino acid sequences SEQ ID NO:1, SEQ ID NO:2, or the nucleotide sequences according to SEQ ID NO:3, SEQ ID NO:4, fragments thereof, and / or mixtures thereof, are in concentrations of between  $10^{-10}$  to  $10^{-5}$  molar (M), more preferably between  $5 \times 10^{-9}$  to  $10^{-6}$  M, most preferably between  $5 \times 10^{-9}$  and  $10^{-7}$  each one.

In a more particular optional embodiment, the peptides of the invention according to the amino acid sequences SEQ ID NO:1, SEQ ID NO:2, or the nucleotide sequences according to SEQ ID NO:3, SEQ ID NO:4, fragments of them, and / or mixtures thereof, when included in a food matrix may be coupled with appropriate molecules, so as to improve its absorption. Such molecules can be selected from but not limited to, polymers such as polyethylene glycol (PEG), chitosan of various molecular weight.

In an even more particular embodiment of the third aspect of the present invention, the application of the prophylactic formulation of the invention is performed in a culture tank, where a dip is given to fish fry that could potentially come into contact with IPNV.

In a more specific embodiment, the application of the prophylactic formulation of the present invention is applied in concentrations between  $10^{-4}$  M to  $10^{-10}$  M, more preferably between  $2 \times 10^{-4}$  M to  $10^{-8}$  M, more preferably between  $8 \times 10^{-4}$  M to  $10^{-6}$  M, even more preferably between  $10^{-5}$  to  $5 \times 10^{-5}$  M.

In a more specific embodiment, the density of fry in the culture tank during the application of the prophylactic formulation of the invention is such to allow the proper development of the fry. For example, the invention considers densities from 10 kg to 70 kg, more preferably between 15 kg and 50 kg, more preferably between 20 kg and 40 kg, more preferably between 25 and 30 kilograms of fry per cubic meter of water in the tank during application of the prophylactic formulation of the invention.

In a particular embodiment of the invention, the fry are exposed to the prophylactic formulation of the present invention for a period of between 1 and 24 hours, more preferably between 2 and 15 hours, more preferably between 4 and 10 hours, more preferably for at least 6 hours.

The invention is illustrated below based on various laboratory tests (*in vitro*) and pilot-scale (*in vivo*). The examples provided below are not intended to limit the scope of the invention but to provide sufficient information so that a person skilled in the art may understand.

## EXAMPLES

The following examples are provided to illustrate some aspects of the invention, without intending to limit its scope, which ultimately is given by the accompanying claims.

To demonstrate the effective entry of the peptides to the cell line, the peptides were labelled with rhodamine-B, and the entry of the peptides into cells evaluated via fluorescence microscopy. Subsequently the cytotoxic capacity of the peptides were evaluated by Trypan Blue and MTT. Together with this, virus expression was determined by quantitative analysis using the technique of "real-time reverse transcription PCR". In order to obtain quantitative results on the inhibition of the infectivity and to calculate viral particle titers in both infection models semiquantitative immunofluorescence will be performed.

Added to this, using continuous sucrose velocity gradients, the profile or polysomal distribution of cultures infected with IPNV subjected to the peptide and without the peptide was evaluated.

It is expected that some of the designed peptides having interfering activity against infection by IPNV, will be used as a basis for the generation of a new prophylactic product for the salmon industry.

### EXAMPLE 1

#### *in vitro* assay 1

##### ***Fluorescent Labeling of Peptides and Fluorescence Microscopy***

Aliquots of the peptides of the invention were labeled with rhodamine-B (0.2 ml of concentration 2.5mg/ml) and stored at -20 °C in the dark until further use. Briefly, exponentially growing Chinook salmon embryo cells (CHSE-214) were exposed to 100ng/well concentration of rhodamine-B labeled peptide p20 for 3 hours on 24 well plates. For direct detection of labeled peptides, the culture medium was discarded and the cells were washed once with PBS (pH 7.3), subsequently rinsed three times with PBS-Tween 20 (0.05%) and analyzed under a Nikon Eclipse 400 fluorescence microscope and recorded with a Nikon Coolpix 4500 digital camera.

##### ***Cells and Cell Culture***

CHSE-214 cells and IPNV-persistently infected cells (10, 19) were cultured as exponentially growing sub confluent monolayers on 24 well plates in MEM and Leibovitz (L-15) medium respectively, supplemented with 10% (v/v) fetal calf serum (FCS) and 2 mM glutamine and maintained.

***IPNV growth and titration***

The virus growth and titration were evaluated on two models of infection: fresh CHSE-214 cells infected *de novo* and the established CHSE-214-NV1015 cell line persistently infected with IPNV. For *de novo* infections, the virus propagated on CHSE-214 cell monolayers were infected at semi confluency at a multiplicity of infection of 0,001 in Leibovitz's medium (L-15, Gibco) at 18°C, supplemented with 50µM gentamicyn, 2mM L-glutamine and 10% fetal bovine serum (FBS, Gibco). After full cytopathic effect was observed, culture fluids were harvested and titered, quantifying the number of infective particles, using a modified Reed and Muench protocol (10, 24). The maintenance medium (MM) was identical except that the serum concentration was reduced to 2%.

***p20 inhibitory potential in de novo infected cells.***

The putative antiviral activity of p20 was measured in *de novo* infected cells followed by indirect immuno detection. CHSE-214 cells were grown on 24 well cell culture plates to confluency and infected with IPNV at a multiplicity of infection of one (MOI = 1). 18 hours prior to infection (p.i.) the cells were washed and the peptide added at 10µM, the concentration at which in our hands full inhibition was attained for similar peptides (data not shown). The plates were fixed with methanol:acetone (3:1) for 30 min at -20°C washed and incubated with a commercial polyclonal anti VP2-VP3 (BiosChile, Chile) as the first antibody 1/80 for 1 hour at room temperature, washed and followed by an anti-rabbit FITC-conjugated antibody (Fluorotest, BiosChile). The modified semi quantitative method of Reed and Muench (10, 24), was used to determine the focal fluorescent units under a fluorescent microscope. The distribution of the fluorescence was analyzed on a Nikon Eclipse 400 fluorescence microscope equipped with a 100-watt mercury lamp. Color photography was performed with a Nikon Coolpix 4500 digital camera.

***p20 inhibitory potential in persistently infected cells***

CHSE-214-NV1015 cells persistently infected with IPNV were grown on 24 well cell culture plates until confluence, the peptide was added at a total concentration of 10µM for four hours, washed and fresh medium added. After 24 hours of peptide treatment total RNA was extracted by the Trizol procedure and VP2 mRNAs expression measured by the RT-qPCR real time procedure (Stratagene 1 step RT-qPCR Kit) with SNPF primers 5'-CAA CAG GGT TCG ACA AAC CAT AC-3' and SNPR primer 5'-TTG ACG ATG TCG GCG TTT C-3'. The reaction was carried out in 30 µl mixture consisting of Brilliant® II SYBR® Green QRT-PCR Master Mix Kit, 1-Step (Stratagene, Inc.), primers and template RNA. Samples were amplified and detected using a Chromo 4 system (BioRad). The final thermo cycling profile was 50°C for 55 minutes, 94°C for 10 minutes and 94°C for 30 s, 55°C for 30 s and 72°C for 40 cycles. 800 ng of total

RNA per experimental sample were used in each triplicate reaction. In order to quantify VP2 mRNA copies of IPNV, a standard curve for DNA quantification was established. To have IPNV real-time PCR standards, the VP2 region was amplified using published procedures standardized in our laboratory. The VP2 amplicon was cloned into PCR 2.1 vector (Invitrogen Inc.) and its specificity confirmed by sequencing. Plasmid DNA was isolated using the Quiaprep miniprep kit (Quiagen) according to the manufacturer's instructions. The purified plasmid was quantified using a Nanodrop device (Nanodrop ND-1000) and serially diluted in Sigma DNase-RNase free water to a final concentration ranging from 10 to  $1 \cdot 10^5$  to 10 copies of genome-equivalents/2 $\mu$ L. Two microliters of each dilution were used for real-time PCR in triplicates to create a standard curve to be used to quantify putative IPNV DNA amounts in experimental samples. (Figures 1 and 2)

### ***Cytotoxicity assay***

The putative toxic effect of the synthetic peptide p20 on eukaryotic cells was measured by exposing established CHSE-214 cells to the peptide according to standard procedures developed in our laboratory (5, 29). Briefly, cell monolayers at 70% semi confluence were washed with PBS and then the peptide p20 added at a range of concentrations (1–100 mM) in triplicate wells and incubated for the maximum viability time (3 h) without culture medium. Samples were then washed three times with excess of PBS before adding 0.1% trypsin in the presence of EDTA for 30–60 s to release cells from the monolayer. Individual cell viability was determined using the Trypan Blue exclusion technique .

## **EXAMPLE 2**

### ***in vitro assay 2***

#### **I. In vitro evaluation of the potential of peptide 182 in the reduction of the expression of new IPN virus particles.**

To evaluate the effectiveness of peptide 182 in the reduction in the expression of new viral particles in CHSE-214 cells infected with IPNV, two experimental situations designated pre-infection treatment and post-infection treatment were designed and evaluated. Both experimental situations were evaluated by two different processes, which correspond to relative quantification of the VP2 gene and determination of viral titer by counting focal fluorescence units.

#### **Treatment pre infection**

To assess the ability of peptide 182 in reducing the production of new virus particles in an *in vitro* model of infection, CHSE-214 cells were pretreated with decreasing molar concentration of peptide 182, from  $1 \cdot 10^{-4}$  to  $1 \cdot 10^{-10}$ M, determined in the experiments on entry of rhodamine-

labeled peptide and peptide 182 toxicity tests on CHSE-214 cells.

To conduct this test, CHSE-214 cells at  $0.8 \times 10^4$  cells / ml, were seeded in two 24 well plates. 24 hours after sowing, the cells were treated for 4 hours with 200uL of peptide 182 in molar concentrations. As in the previous cases, the peptide was solubilized in L-15 medium without serum. After treatment with peptide 182, the cells were washed 2 times with 1x PBS and subsequently infected with an MOI of 1x IPNV which had a titer of  $7.6 \times 10^7$ . Inoculation with the virus was carried out for 1 hour at 17 ° Celsius, followed by a gentle washing with 1x PBS. Maintenance of the infected cells was performed with 1 ml of L-15 medium supplemented with 2% SFB at 17 ° C for 16 hours post infection (hpi). (Figure 3)

#### **Treatment Post infection**

To determine the effectiveness of the peptide in reducing the number of new IPN virus particles CHSE-214 cells were seeded and infected according the previously described protocols. Post-infection and after washing with 1x PBS, cells were incubated with decreasing molar concentrations of peptide 182 from  $10^{-4}$  to  $10^{-10}$  M for 4 hours, the peptide was removed, the cells were washed with 1 x PBS and kept in 1 ml of L-15 medium with 2% SFB for 16 hpi (Figure 4).

#### **Evaluation of IPNV titre reduction by focal fluorescence.**

To evaluate the effectiveness of peptide 182 in the reduction of viral particles in pre-infection and post-infection conditions, the expression of the VP2 protein was measured by quantifying relative to elongation factor ELF-1 alpha of the CHSE-214 cells using RNA extraction and real-time PCR described in methodology of objective 2. The reduction in viral titre was evaluated using the procedure described in the literature and mentioned above, assessment in this case only was performed using a commercial monoclonal antibody against VP2 and with decreasing concentration of the peptide from  $10^{-4}$  to  $10^{-8}$ M.

### **RESULTS 1**

***In vitro* evaluation of the potential of peptide 182 in reducing the expression of new IPN virus particles.**

**II. Design of a model of controlled *in vivo* challenge with IPNV to evaluate the effectiveness of peptide 182 in reducing the mortality of infected *Salmo salar* fry.**

#### **Cultivation system**

A unit was designed with 5 culture systems (experimental lines) with independent recirculating fresh water. Each culture system is composed of two culture units with a maximum capacity of 20 liters each, the system consists of a trickling 25L biofilter for the removal of solids and nitrogen compounds and a drain tank of 200L for the removal of residual water and feces. The

water removed from the tanks post circulation is treated in the drain with ozone (O<sub>3</sub>) and UV light to remove contaminants before returning to the sewer system, ensuring the removal of the remnants of the challenge virus.

#### **Individuals and culture conditions.**

For the experimental challenge of Atlantic salmon (*Salmo salar*), fry were purchased from an Invertec fish farm in Curacautin, Araucania region. 5000 individuals with an average weight of 1.4g were transferred to the premises of the Laboratory of Aquaculture Engineering, Catholic University of the Holy Conception (UCSC) where they were acclimated for 11 days prior to the experiment.

After the acclimatization, fry were separated into groups of 250 individuals per unit area, there were a total of 500 fry per culture system (experimental line). The culture conditions used were similar to those used in industrial fish farms, ensuring that these said conditions did not affect the development of individuals nor influence the results of the challenge. The general conditions correspond to a density of 25kg/m<sup>3</sup> culture, with cycles of 16 hours light and 8 hours dark. Each tank was oxygenated in a ratio of 7 mg / L and the rate of water exchange in the system was 250 ml per minute, where the total turnover is within 4 hours.

**Table 1: Conditions of culture production used in the *in vivo* challenge in the wet lab at UCSC.**

| <b>Culture conditions</b>            | <b>Characteristic value</b> | <b>Unit of measurement</b> |
|--------------------------------------|-----------------------------|----------------------------|
| <b>Water management</b>              |                             |                            |
| <b>Recirculation rate</b>            | 4                           | times/hour                 |
| <b>Renewal of fresh water</b>        | 100                         | % daily                    |
| <b>Turnover rate</b>                 | 15                          | cms/seg                    |
| <b>Production</b>                    |                             |                            |
| <b>Initial weight of individuals</b> | 1.4                         | grs.                       |
| <b>N° of individuals per unit</b>    | 250                         | individuals                |
| <b>Culture density</b>               | 25                          | Kg/m <sup>3</sup>          |
| <b>Feed (P.C)</b>                    | 2,5                         | %                          |
| <b>Water quality</b>                 |                             |                            |
| <b>Ammonia nitrogen</b>              | ≤ 1                         | mg/L                       |
| <b>Nitrite</b>                       | ≤ 0.5                       | mg/L                       |
| <b>pH</b>                            | 6.5 ≥ ≤ 7.5                 | mg/L                       |
| <b>Dissolved oxygen</b>              | ≤ 10                        | mg/L                       |
| <b>Total suspended solids</b>        | 40 ~ 80                     | mg/L                       |

### Initial evaluation of the *Salmo salar* fry

Before the challenge, a sample of 50 individuals was analyzed to determine the status of the fish and previous infection with IPNV. As described above, the persistence of the virus is one of the most common conditions for cultures such as salmon species.

The preliminary screening was performed using VP2SNP-F and VP2SNP-R primer set using the protocols for real-time PCR previously described in Methods.

### *In vivo* challenge model on medium scale. Wet Laboratory Model.

The experimental evaluation was conducted for 56 days, after the acclimatization of the fry. The application of peptide 182 was performed in a bath by reduction of the water column. For the challenge the VR- 299 strain of IPNV obtained from cell cultures of CHSE-214, was used, as described previously in Methods. The virus was titered by IFAT at around  $7.6 \cdot 10^7$  particles / ml. For the challenge, the water column was reduced, leaving the *Salmo salar* fry at a density of 50 kg/m<sup>3</sup>. Two types of treatment with the peptide described as pre- challenge treatment and post-challenge treatment according to the similar condition used in the *in vitro* model were performed. Two culture systems were used as a control, a peptide application control and the other an IPNV infection control, a final system was used as control of natural mortality. Final concentration of virus used in the challenge was  $10^5$  viral particles per ml, and the peptide concentration used was  $10^{-5}$ M, the molar concentration was selected based on *in vitro* results. Table 2 summarizes the experimental details.

**Table 2: Sample experimental design with peptide 182 (p182). Every situation has two separate tanks with 250 *Salmo salar* fry, average weight 1.4 g.**

| System                       | Step 1                                                     | Step 2<br>1 hour                                          | Spet 3                                                                   | Step 4                                                    |
|------------------------------|------------------------------------------------------------|-----------------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------|
| <b>Pre-challenge</b>         | Bath with p182 for 4 hours.<br>Density 50kg/m <sup>3</sup> | Normal culture conditions<br>Density 25Kg/m <sup>3</sup>  | Bath with IPNV for 3 hours. Density 50kg/m <sup>3</sup>                  | Normal culture conditions.<br>Density 25Kg/m <sup>3</sup> |
| <b>Post-challenge</b>        | Bath with IPNV for 3 hours. Density 50kg/m <sup>3</sup>    | Normal culture conditions<br>Density 25Kg/m <sup>3</sup>  | Bath with p182 for 4 hours. Density 50kg/m <sup>3</sup>                  | Normal culture conditions<br>Density 25Kg/m <sup>3</sup>  |
| <b>Negative Control p182</b> | Bath with p182 for 4 hours.<br>Density 50kg/m <sup>3</sup> | Normal growing conditions.<br>Density 25Kg/m <sup>3</sup> | Reduced water bath conditions for 3 hours<br>Density 50kg/m <sup>3</sup> | Normal growing conditions<br>Density 25Kg/m <sup>3</sup>  |

|                              |                                                               |                                                        |                                                                        |                                                        |
|------------------------------|---------------------------------------------------------------|--------------------------------------------------------|------------------------------------------------------------------------|--------------------------------------------------------|
| <b>Positive control IPNv</b> | Bath with IPNv for 3 hours. Density 50kg/m <sup>3</sup>       | Normal growing conditions. Density 25Kg/m <sup>3</sup> | Reduced water bath conditions for 4 hours. Density 50kg/m <sup>3</sup> | Normal growing conditions. Density 25Kg/m <sup>3</sup> |
| <b>Water Control</b>         | Reduced water column for 4 hours. Density 50kg/m <sup>3</sup> | Normal growing conditions. Density 25Kg/m <sup>3</sup> | Reduced waterbath conditions for 3 hours. Density 50kg/m <sup>3</sup>  | Normal growing conditions. Density 25Kg/m <sup>3</sup> |

## RESULTS II

**Designing a challenge model of controlled *in vivo* infection with IPNv to evaluate the effectiveness of peptide 182 in reducing the mortality of infected *Salmo salar* fry.**

### **Initial evaluation of *Salmo salar* fry**

The initial state of the *Salmo salar* fry with respect to preexisting conditions, was determined by an initial sampling which indicated that 60% of sampled fish were found to have IPN viral load. This is consistent with the literature which explains that the virus becomes persistent in fish that survive an outbreak and therefore the offspring may be asymptomatic carriers of the virus. Because of this, the untreated tank was considered to represent the natural mortality rate which may exist due to the activation of virus in the experimental tanks and the handling of the water column in which they were maintained. (Figure 9).

### ***In vivo* challenge model on medium scale. Wet laboratory model.**

Using the previously detailed conditions, *in vivo* testing on wet laboratory scale was performed. Experimental evidence is presented below as well as graphs showing the daily mortalities in the culture systems designed, as well as the accumulated mortality rate calculated after the termination of the experiment (Figure 10).

A test was performed in conditions similar to those used in the previous examples.

The peptide p182 was applied by bath immersion to 300,000 fry with the company Marine Harvest. Samples were taken at 0, 30 and 60 days of application of the peptide and 25 specimens were analyzed.

The results obtained in this study are summarized in the two graphs in Figure 11.

## EXAMPLE 4

**Evaluation of Peptides interfering with IPNv incorporated into the diet of *Salmo salar* fry persistently infected with the virus**

**OBJECTIVE:** To determine whether interfering peptides, already tested for their inhibitory effect on viral load "*in vivo*", are able to induce the same effect via feed.

The evaluation of the interfering IPNV peptides incorporated into food consisted of two stages.

**FIRST STAGE:** developed at the experimental station in Quillaipe in collaboration with Aquadvice, where five diet formulations previously prepared by oil bath with interfering peptides p182; p20 and the mixture of both to use as an unrelated control (Table 3) were applied to a total of 1000 specimens of *Salmo salar* per formulation with an initial average weight of 13 grams which increased to an average of 20 grams during the 45 days of the peptide experiment. There were no significant differences in body weight gain between the fish fed with the different formulations (Table 4).

The fish were distributed in 5 tanks of 40 fish each per formulation (Table 5). Environmental parameters were measured in each tank (temperature in ° C and % oxygen saturation) daily for 45 days of the trial, which remained constant throughout the time.

During the 45 days of the trial there were 5 mortalities, not associated IPNV infection.

To assess the initial viral load of fish analyzed, 30 were selected to determine the degree of infection with the following agents: IPNV (56.6%); ISAv (0%); *P. salmonis* (0%).

For the assessment liver, kidney and heart of each fish was sent to GIM-PUCV individually preserved in RNA later ®.

**SECOND STAGE:** developed at IMT-PUCV. To determine the viral load of fish analyzed, 180 untreated fish were taken at time "0" and the variation in Ct values was quantitatively determined in reference to the ratio of viral VP2 gene / cellular ELF. Four time points were analyzed in the experiment (days 10, 20, 30 and 45), removing 25 fish per formulation for which the ratio was Ct Vp2/ELF was determined in duplicate except for day 45 when 25 fish per formulation were tested without duplicates, in a pool of organs per fish of which 30 mg were taken to extract RNA with E.Z.N.A. kit for qRT-PCR.

## SUMMARY OF RESULTS

The results indicate:

- 1.- That the average viral load decreases sequentially with time in fish fed with interfering peptides
- 2.- That the range of Ct values becomes smaller over time in fish treated with interfering peptides
- 3.- The above effect is more significant in the synergy between peptides p182 and p20 (Formulation 4)
- 4.- Although there is a reducing effect of the control peptide, the two specific peptides exhibit a higher level of attenuation.

**Table 3: Formulations of the IPNV interfering peptides incorporated in the feed**

|                      | <b>Molarity</b>                     | <b>FORMULATION</b>   |
|----------------------|-------------------------------------|----------------------|
| <b>Formulation 1</b> | $2 \cdot 10^{-7}$                   | Unrelated peptide    |
| <b>Formulation 2</b> | $2 \cdot 10^{-7}$                   | Peptide 182          |
| <b>Formulation 3</b> | $2 \cdot 10^{-9}$                   | Peptide 19           |
| <b>Formulation 4</b> | $2 \cdot 10^{-7} + 2 \cdot 10^{-9}$ | Peptide 182+19       |
| <b>Formulation 5</b> | 0                                   | Feed without peptide |

**Table 4: Variation in the body weight of the fish analyzed**

| Weight gain per treatment from initial sampling to final sampling (day 45) |               |                 |                             |                     |               |                           |                   |          |         |                                 |                                     |
|----------------------------------------------------------------------------|---------------|-----------------|-----------------------------|---------------------|---------------|---------------------------|-------------------|----------|---------|---------------------------------|-------------------------------------|
| Treatment                                                                  | Tanks         | initial N° fish | average weight at day 0 (g) | initial biomass (g) | final N° Fish | average weight day 45 (g) | Final biomass (g) | Growth % | SGR (%) | daily weight gain of a fish (g) | weight gain in 45 days per tank (g) |
| A                                                                          | 3-8-11-16-23  | 200             | 12.9                        | 2580.16             | 124           | 20.03                     | 2483.8            | 55.27    | 0.98    | 0.158                           | 7.13                                |
| B                                                                          | 4-9-12-17-24  | 200             | 13.0                        | 2600.66             | 120           | 20.18                     | 2421.5            | 55.18    | 0.98    | 0.159                           | 7.18                                |
| C                                                                          | 5-10-13-18-25 | 200             | 12.76                       | 2551.90             | 124           | 19.31                     | 2394.6            | 51.35    | 0.92    | 0.146                           | 6.55                                |
| D                                                                          | 2-7-14-19-22  | 200             | 12.91                       | 2581.3              | 125           | 20.04                     | 2505.2            | 55.28    | 0.98    | 0.159                           | 7.14                                |
| E                                                                          | 1-6-15-20-21  | 200             | 12.99                       | 2597.1              | 123           | 19.67                     | 2419.5            | 51.48    | 0.92    | 0.149                           | 6.69                                |

**Table 5: Tank distribution for the tests on peptides interfering with IPNV**

| <b>Formulation</b> | <b>Diet</b> | <b>Tanks by diets</b> | <b>N° of fish x tank</b> | <b>N° of fish per Diet</b> |
|--------------------|-------------|-----------------------|--------------------------|----------------------------|
| T - 1              | A           | 3 - 8 - 11 - 16 - 23  | 40                       | 200                        |
| T -2               | B           | 4 - 9 - 12 - 17 - 24  | 40                       | 200                        |
| T -3               | C           | 5 - 10 - 13 - 18 - 25 | 40                       | 200                        |
| T -4               | D           | 2 - 7 - 14 - 19 - 22  | 40                       | 200                        |
| T -5               | E           | 1 - 6 - 15 - 20 - 21  | 40                       | 200                        |

**Table 6: Processing scheme of samples at GIM - PUCV.**

|               |                              |
|---------------|------------------------------|
| <b>Organ</b>  | <b>Heart + Liver+ Kidney</b> |
| <b>Step 1</b> | <b>Pool</b>                  |
| <b>Step 2</b> | <b>30 mg/fish</b>            |
| <b>Step 3</b> | <b>RNA extraction</b>        |
| <b>Step 4</b> | <b>qRT-PCR (VP2/ELF)</b>     |

Store at -80°C

**Table 7: Summary of fish processed (All samples in duplicate)**

| Formulations            | Day 0 | Day 10 | Day 20 | Day 30 | Day 45 |
|-------------------------|-------|--------|--------|--------|--------|
| Peptide control         | -     | 25     | 25     | 25     | 25/125 |
| Peptide 182             | -     | 25     | 25     | 25     | 25/125 |
| Peptide 19              | -     | 25     | 25     | 25     | 25/125 |
| Peptide 182+19          | -     | 25     | 25     | 25     | 25/125 |
| S/peptide               | -     | 25     | 25     | 25     | 25/125 |
| Control                 | 180   | -      | -      | -      | -      |
| Total qRT-PCR reactions | 360   | 250    | 250    | 250    | 125    |

**Table 8: Controls. Persistently infected fish, maximum and minimum values of Ct for the VP2 gene compared to the cell marker ELF.**

|                 | Samples  | Ct minor | Ct minor | Average | Ct major | Ct major | Average |
|-----------------|----------|----------|----------|---------|----------|----------|---------|
| Day 0           | 180 (x2) | 17.64    | 17.38    | 17.5    | 31.13    | 30.97    | 31.05   |
| Initial control | 30 (x1)  | 18.00    | -        | -       | 40.00    | -        | 28.32   |

## INDUSTRIAL APPLICABILITY

The present invention provides peptides that are suitable for pharmaceutical or veterinary compositions, which can help in the prophylaxis of viruses of the *Birnaviridae* family, more particularly, for the prophylaxis of fish that can be exposed to IPNV.

## CLAIMS

1. Composition comprising a prophylactic agent or a therapeutic agent or a prophylactic dietary supplement with therapeutic potential useful in the prevention and treatment of diseases caused by viruses of the family *Birnaviridae*, comprising synthetic peptides which have the property of reducing the infection rate of animals exposed to a virus of the *Birnaviridae* family in particular.
2. Composition comprising a prophylactic agent or a therapeutic agent or a prophylactic food supplement with therapeutic potential useful in the prevention and treatment of diseases produced by viruses of the *Birnaviridae* family, wherein the particular virus of the *Birnaviridae* family is selected among infectious pancreatic necrotic virus (IPNV), yellow tail ascites virus (YTAV), marine birnavirus (MABV), blotched snakehead virus (BSNV), Infectious Bursal Disease virus (IBDV); and *Drosophila X virus*.
3. Composition comprising a prophylactic agent or a therapeutic agent or a prophylactic food supplement with therapeutic potential useful in the prevention and treatment of diseases produced by viruses of the *Birnaviridae* family according to claim 1 or claim 2, wherein the synthetic peptides corresponding to subsequences coding pVP2 protein of the virus capsid.
4. Composition comprising a prophylactic agent or a therapeutic agent or a prophylactic food supplement with therapeutic potential useful in the prevention and treatment of diseases caused by viruses of the *Birnaviridae* family according to claim 3, characterized in that the peptides correspond to peptides with an identity of at least 80% compared to the amino acid sequences SEQ ID NO:1 or SEQ ID NO:2.
5. Composition comprising a prophylactic agent or a therapeutic agent or a prophylactic food supplement with therapeutic potential useful in the prevention and treatment of viral diseases of the *Birnaviridae* family according to claim 3, characterized in that the peptides correspond to peptides with an identity of at least 80% with respect to the nucleotide sequences SEQ ID NO: 3 or SEQ ID NO:4.
6. Composition comprising a prophylactic agent or a therapeutic agent or a prophylactic dietary supplement with useful therapeutic potential useful in the prevention and treatment of diseases caused by viruses of the *Birnaviridae* family according to claim 3, characterized in that it is formulated with excipients that allow the application to a tank of farmed fish that

could potentially come into contact with IPNV.

7. Composition comprising a prophylactic agent or a therapeutic agent or a prophylactic dietary supplement with therapeutic potential useful in the prevention and treatment of diseases caused by viruses of the *Birnaviridae* family according to claim 3, characterized in that it is formulated with excipients that allow it to be added to a food matrix.
8. Composition comprising a prophylactic agent or a therapeutic agent or a prophylactic dietary supplement with therapeutic potential useful in the prevention and treatment of diseases caused by viruses of the *Birnaviridae* family according to claim 7, characterized in that the peptides, fragments of them, and / or mixtures thereof are coupled with appropriate molecules in order to improve absorption.
9. A composition comprising a prophylactic agent or a therapeutic agent or a prophylactic food supplement with therapeutic potential useful in the prevention and treatment of diseases caused by viruses of the *Birnaviridae* family according to claim 8, wherein the molecules are polyethylene glycol (PEG) or chitosan.
10. A method for applying a composition for preventing or treating diseases caused by virus of the *Birnaviridae* family, in particular for prophylactic control of infectious pancreatic necrosis virus (IPNV) comprises applying synthetic peptides in concentrations between  $10^{-10}$  and  $10^{-4}$  M, which have the property of reducing the infection rate of fish exposed to IPNV, where culture water corresponds to the volume of water in the pond where the fry are kept.
11. Method for applying a composition for the prevention or treatment of diseases caused by viruses of the *Birnaviridae* family, in particular for prophylactic control of infectious pancreatic necrosis virus (IPNV) according to claim 10, wherein the peptides correspond to peptides with an identity of at least 80% compared to the amino acid sequences SEQ ID NO:1 or SEQ ID NO:2.
12. Method for applying a composition for the prevention or treatment of diseases caused by viruses of the *Birnaviridae* family, in particular for prophylactic control of infectious pancreatic necrosis virus (IPNV) according to claim 10, wherein the peptides correspond to peptides with an identity of at least 80% with respect to the nucleotide sequences SEQ ID NO:3 or SEQ ID NO:4.
13. Method for applying a composition for the prevention or treatment of diseases caused by viruses of the *Birnaviridae* family, in particular for prophylactic control of infectious

pancreatic necrosis virus (IPNV) according to claim 10, wherein a proper density of fry in the culture tank during application of the prophylactic formulation of the invention is such that the proper development of the fry is permitted.

14. Method for applying a composition for the prevention or treatment of diseases caused by viruses of the *Birnaviridae* family, in particular for prophylactic control of infectious pancreatic necrosis virus (IPNV) according to claim 13, wherein the proper density of fry in the culture tank is in a range from 10 kg to 70 kg of fry per cubic meter of water culture.
15. Method for applying a composition for the prevention or treatment of diseases caused by viruses of the *Birnaviridae* family, in particular for prophylactic control of infectious pancreatic necrosis virus (IPNV) according to claim 10, wherein the fry are exposed to the composition for the prophylactic control for a period of between 1 and 24 hours.
16. A method for applying a composition for preventing production *in vitro* of viral particles of a virus of the *Birnaviridae* family, in particular for prophylactic control of infectious pancreatic necrosis virus (IPNV) comprises applying synthetic peptides in concentrations between  $10^{-10}$  and  $10^{-4}$  M, which have the property of reducing the infection rate of fish cells exposed to IPNV, where culture water corresponds to the volume of water in the medium the cells are kept.
17. A method for applying a composition for preventing production *in vitro* of viral particles of a virus of the *Birnaviridae* family, in particular for prophylactic control of infectious pancreatic necrosis virus (IPNV) according to claim 10, wherein the peptides correspond to peptides with an identity of at least 80% compared to the amino acid sequences SEQ ID NO:1 or SEQ ID NO:2.
18. A method for applying a composition for preventing production *in vitro* of viral particles of a virus of the *Birnaviridae* family, in particular for prophylactic control of infectious pancreatic necrosis virus (IPNV) according to claim 10, wherein the peptides correspond to peptides with an identity of at least 80% with respect to the nucleotide sequences SEQ ID NO:3 or SEQ ID NO:4.
19. A method for applying a composition for preventing production *in vitro* of viral particles of a virus of the *Birnaviridae* family, in particular for prophylactic control of infectious pancreatic necrosis virus (IPNV) according to claim 10, wherein the cells are exposed to the composition for a period of between 1 and 24 hours.

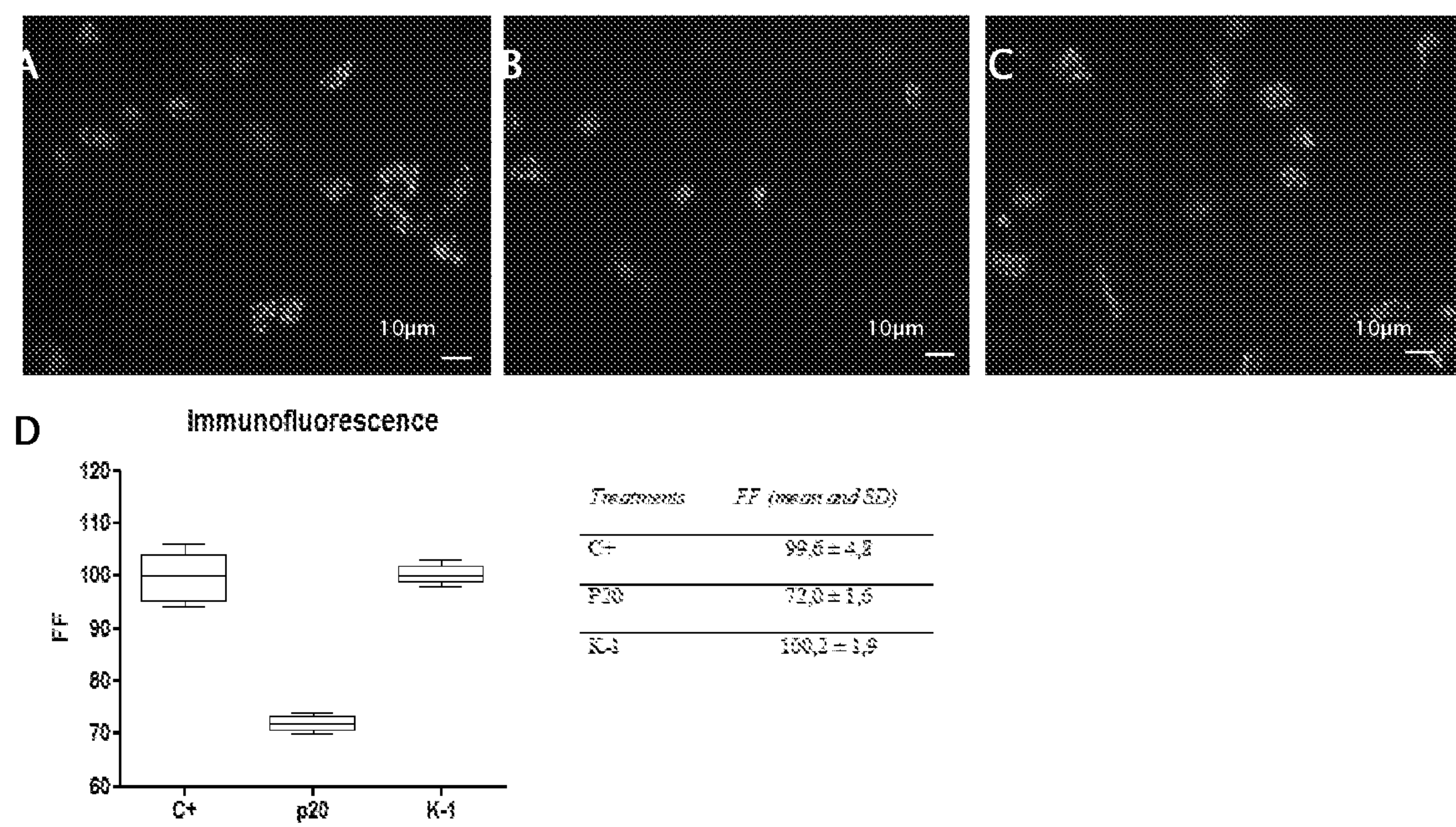


Figure 1

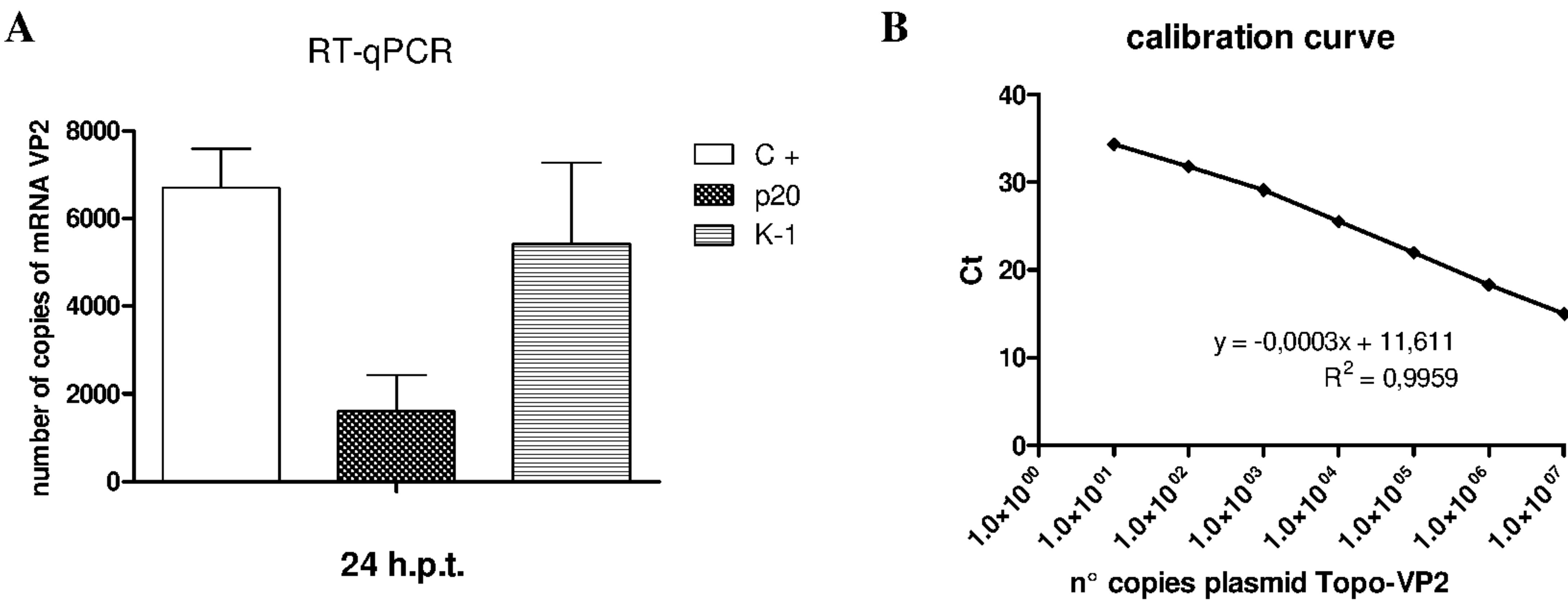


Figure 2

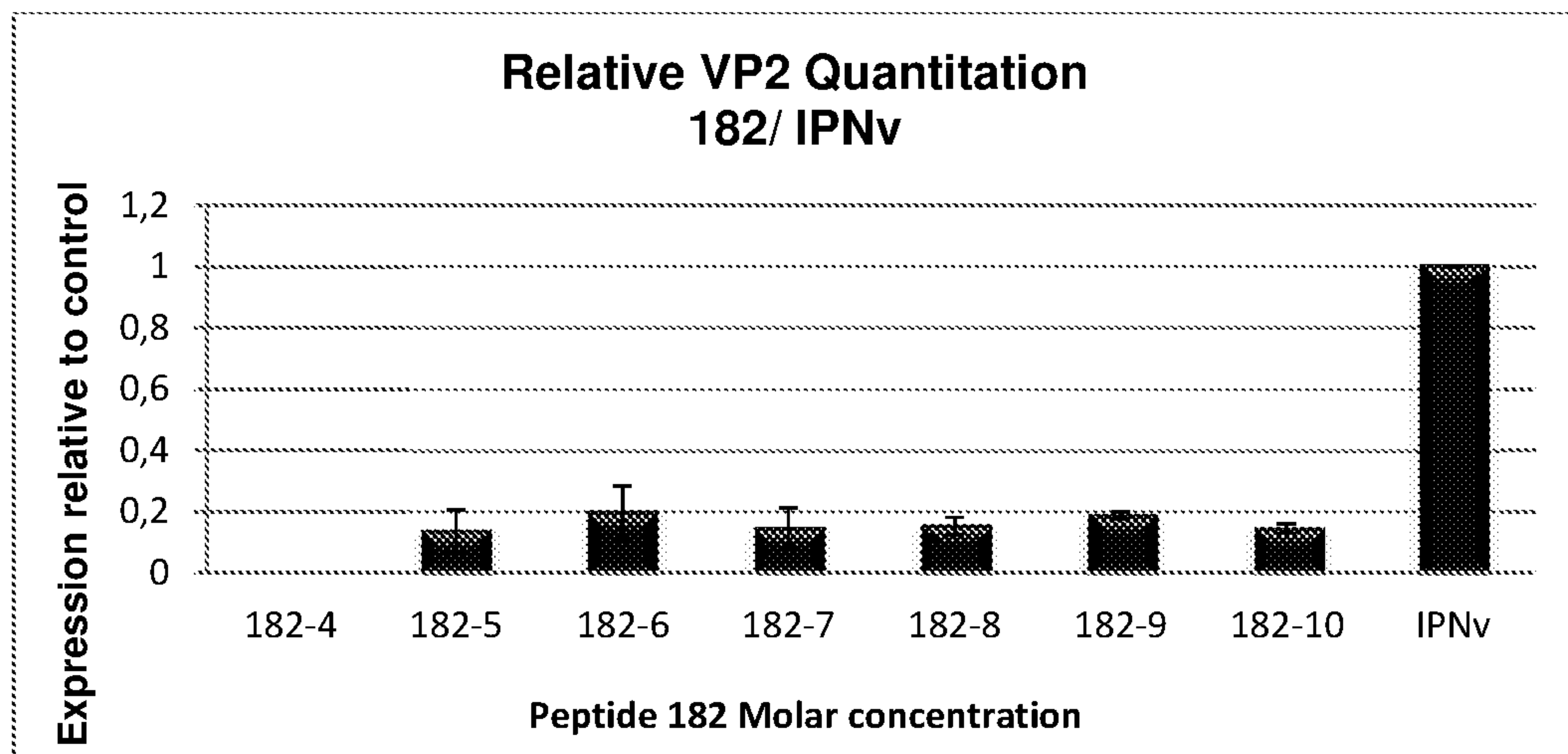


Figure 3

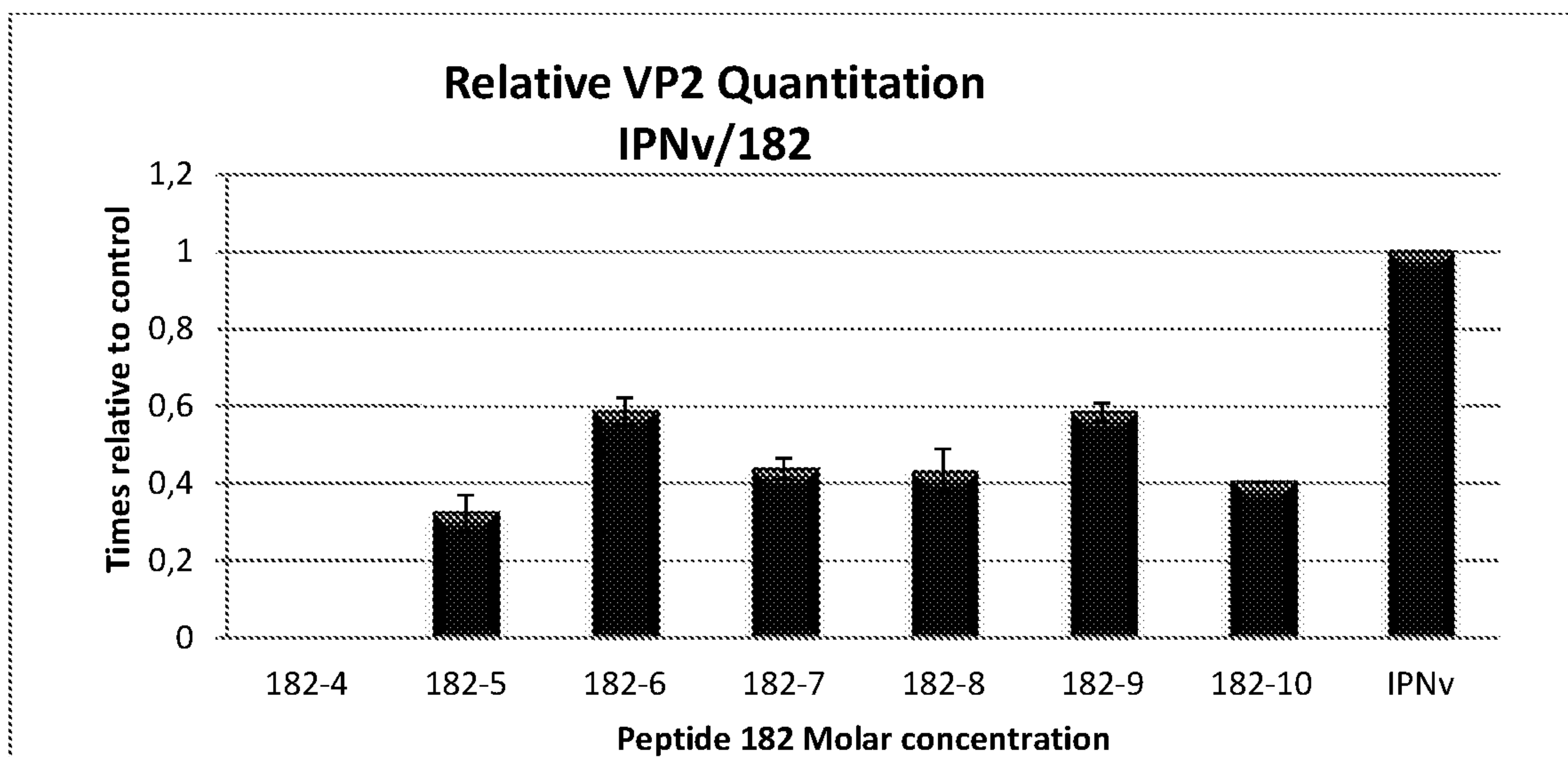


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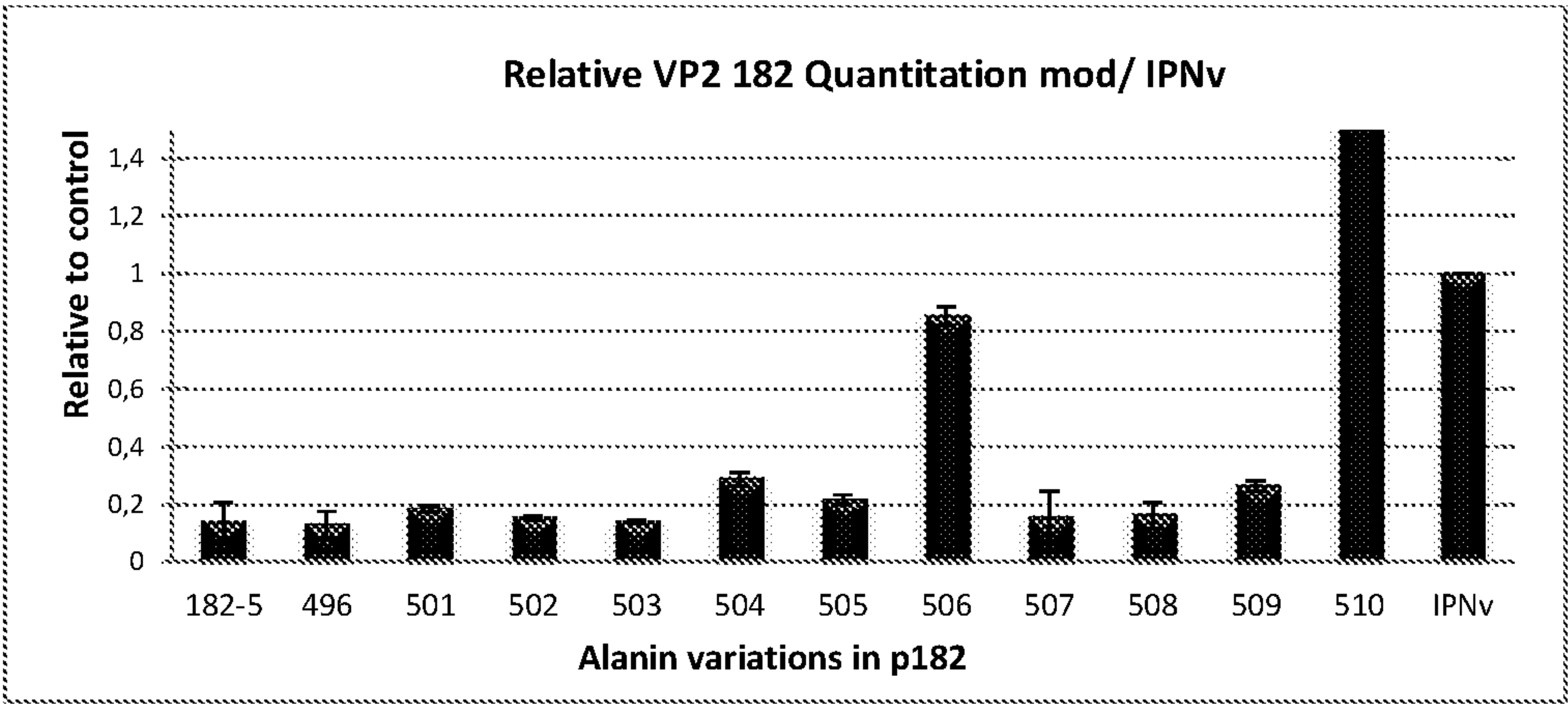


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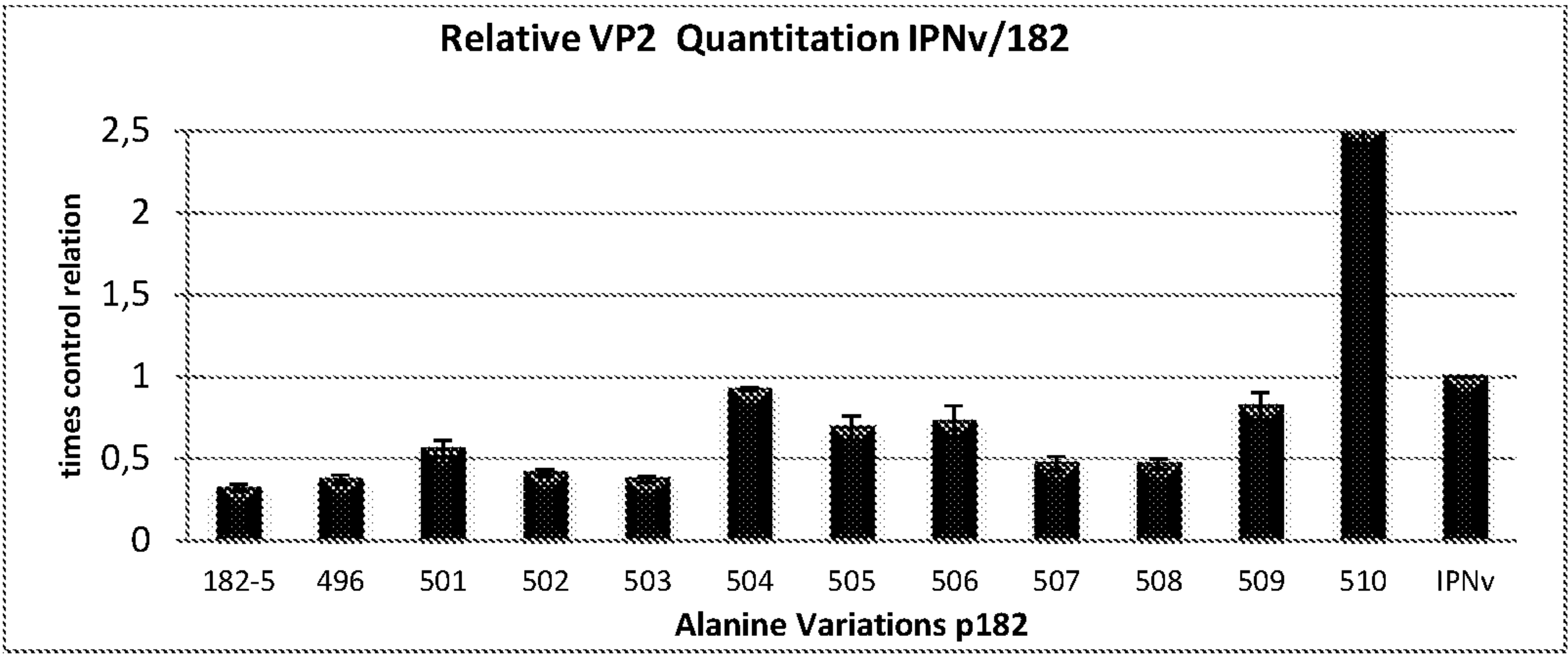
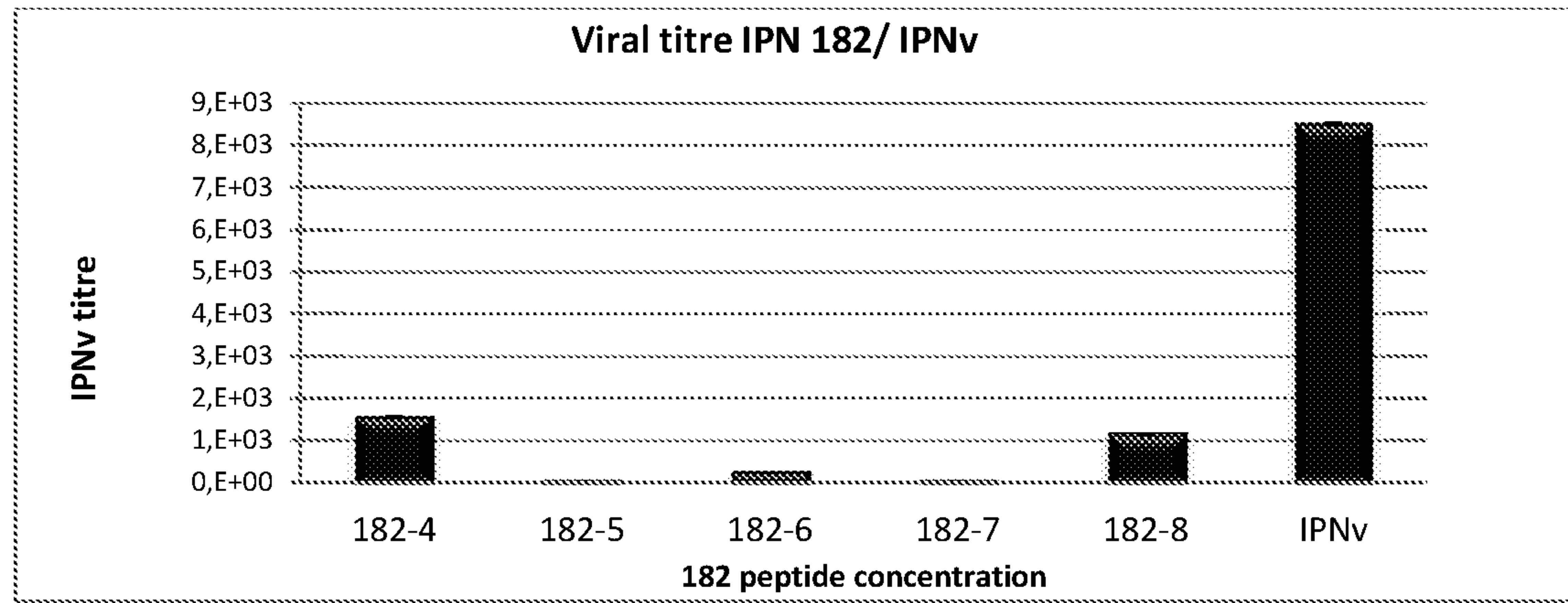
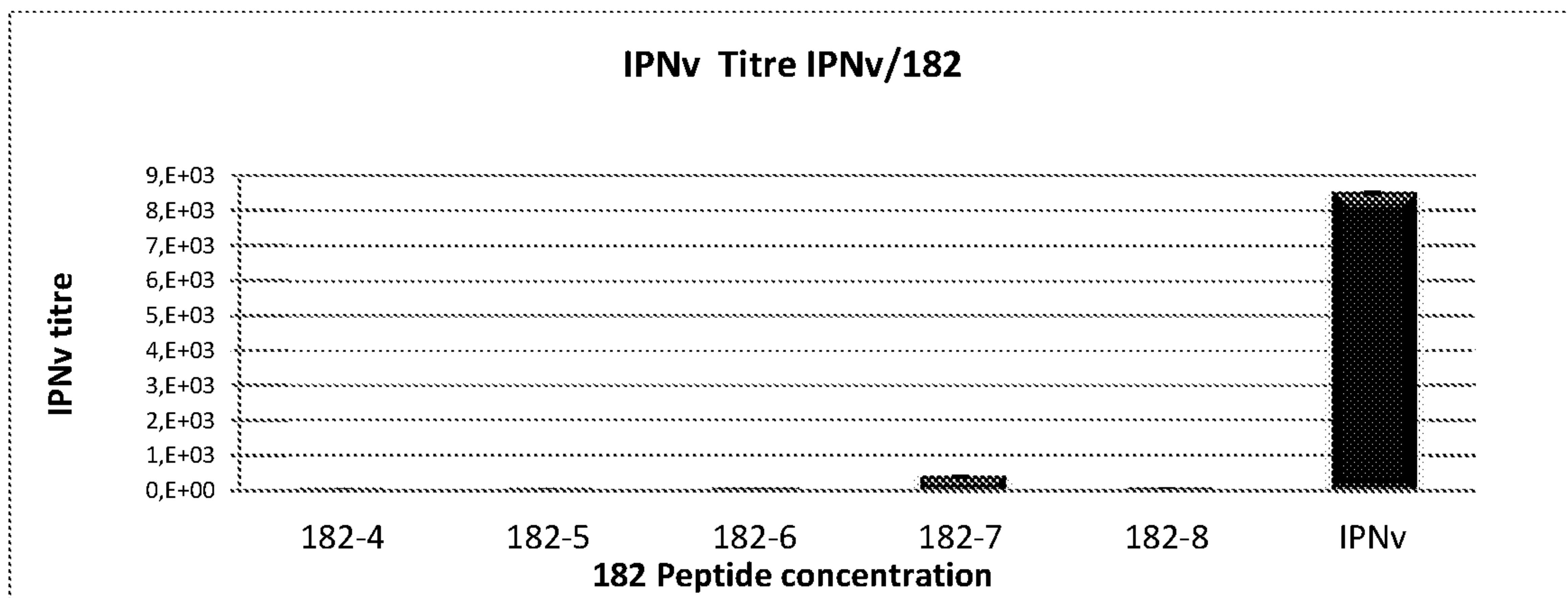


Figure 6

**Figure 7****Figure 8**

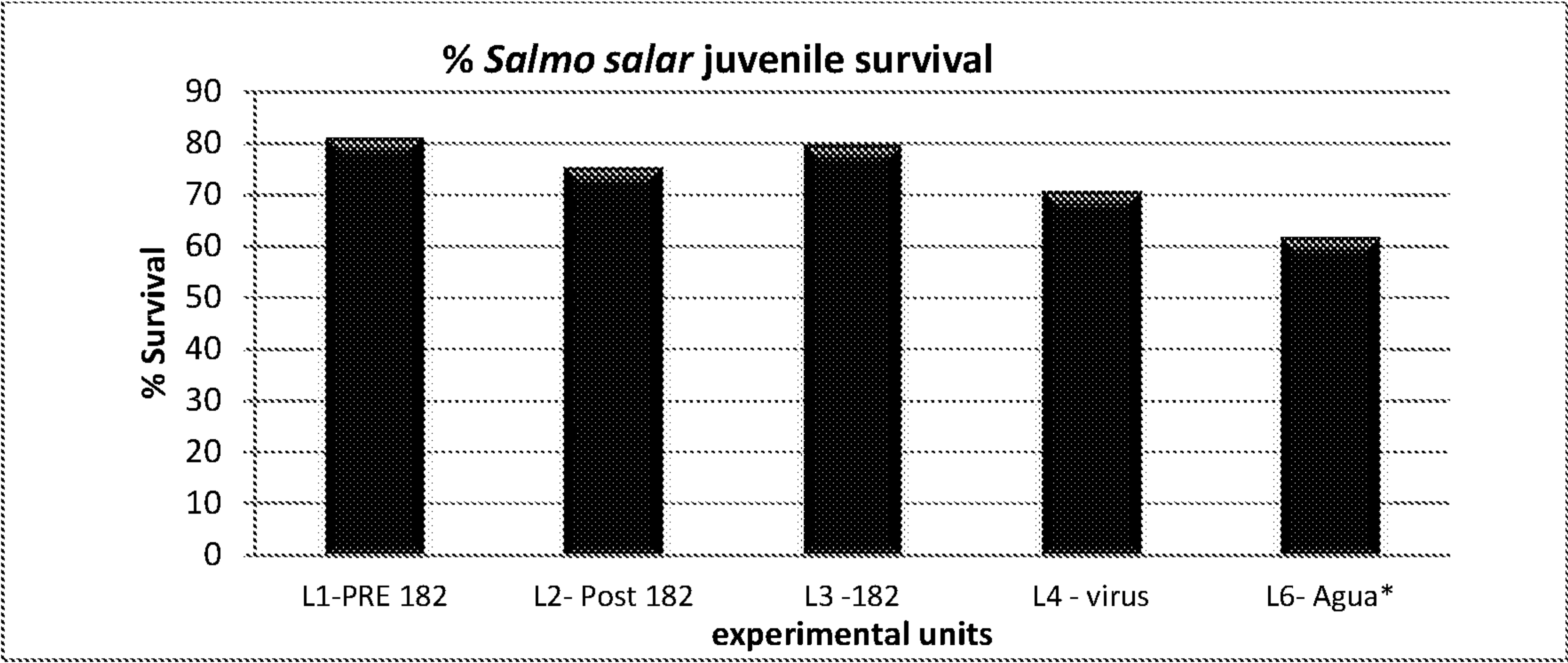


Figure 9

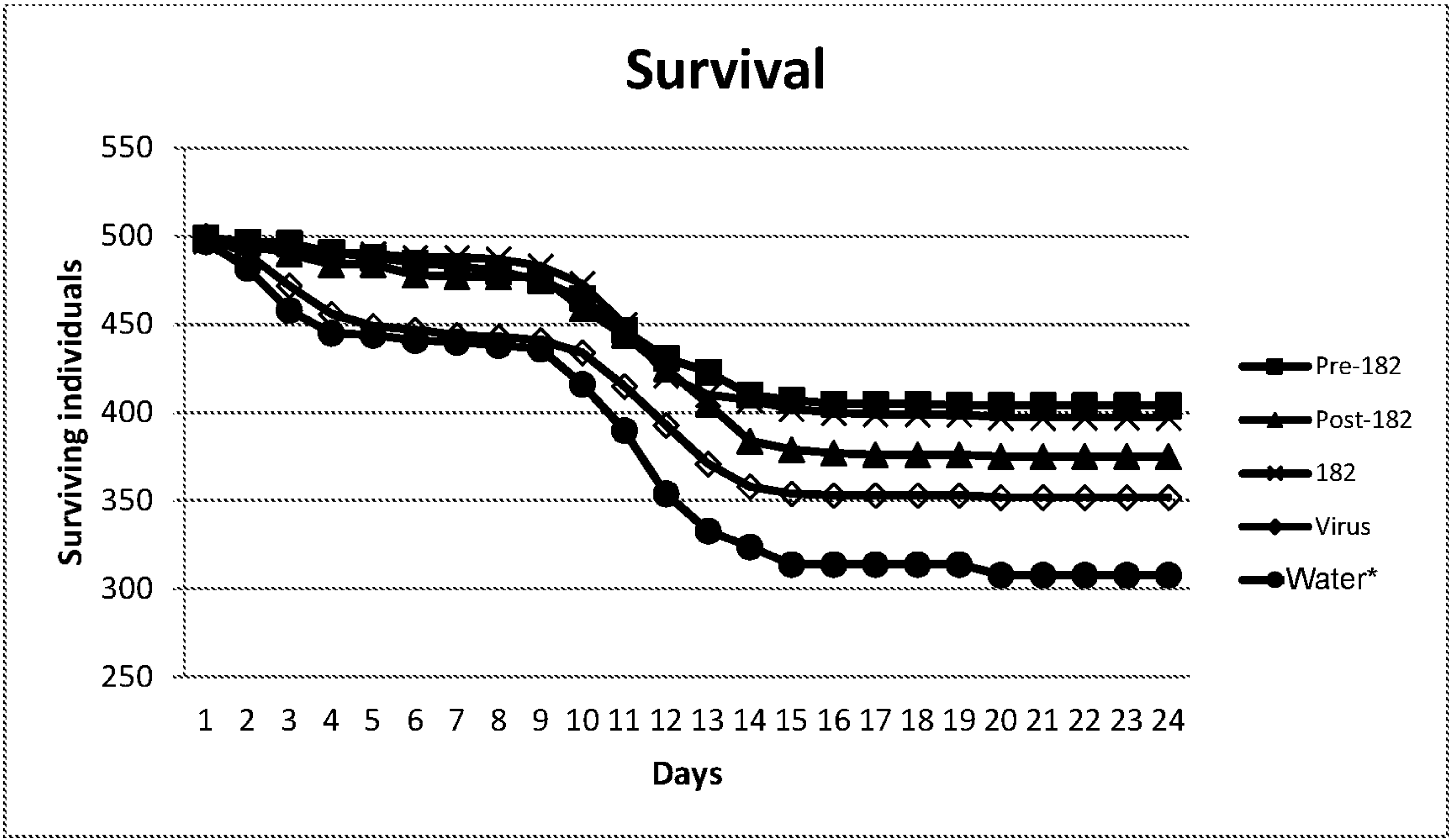


Figure 10

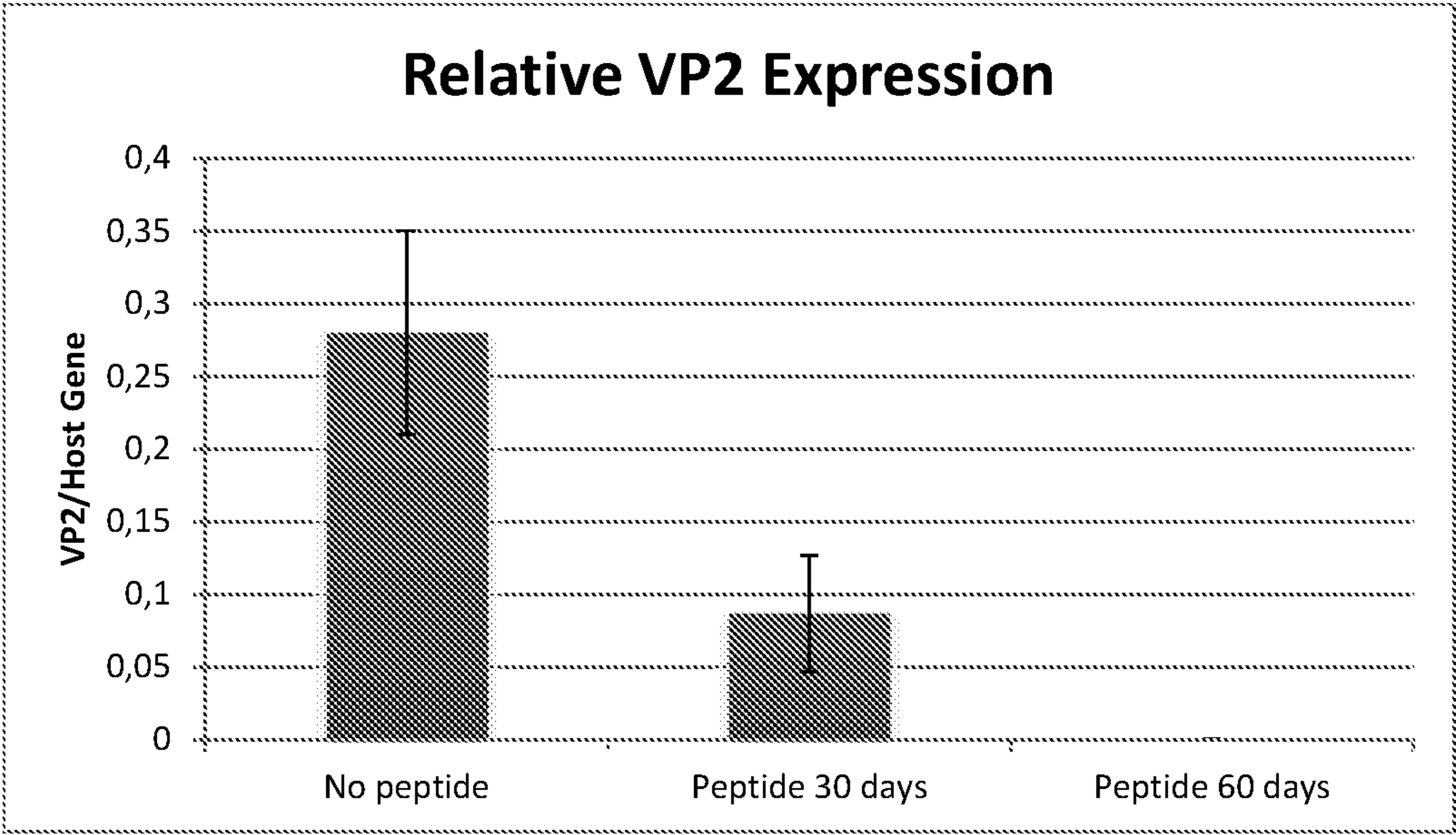
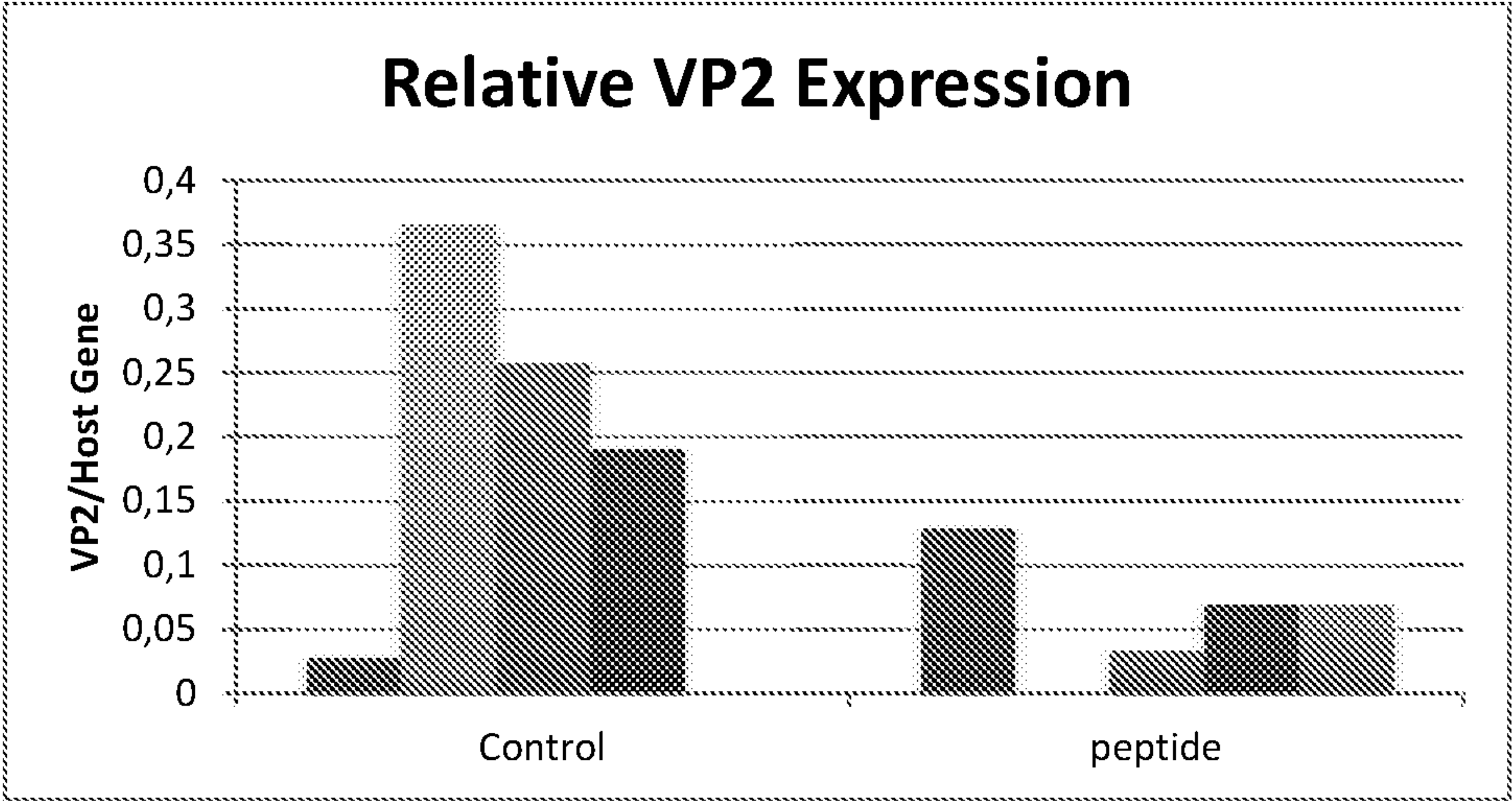
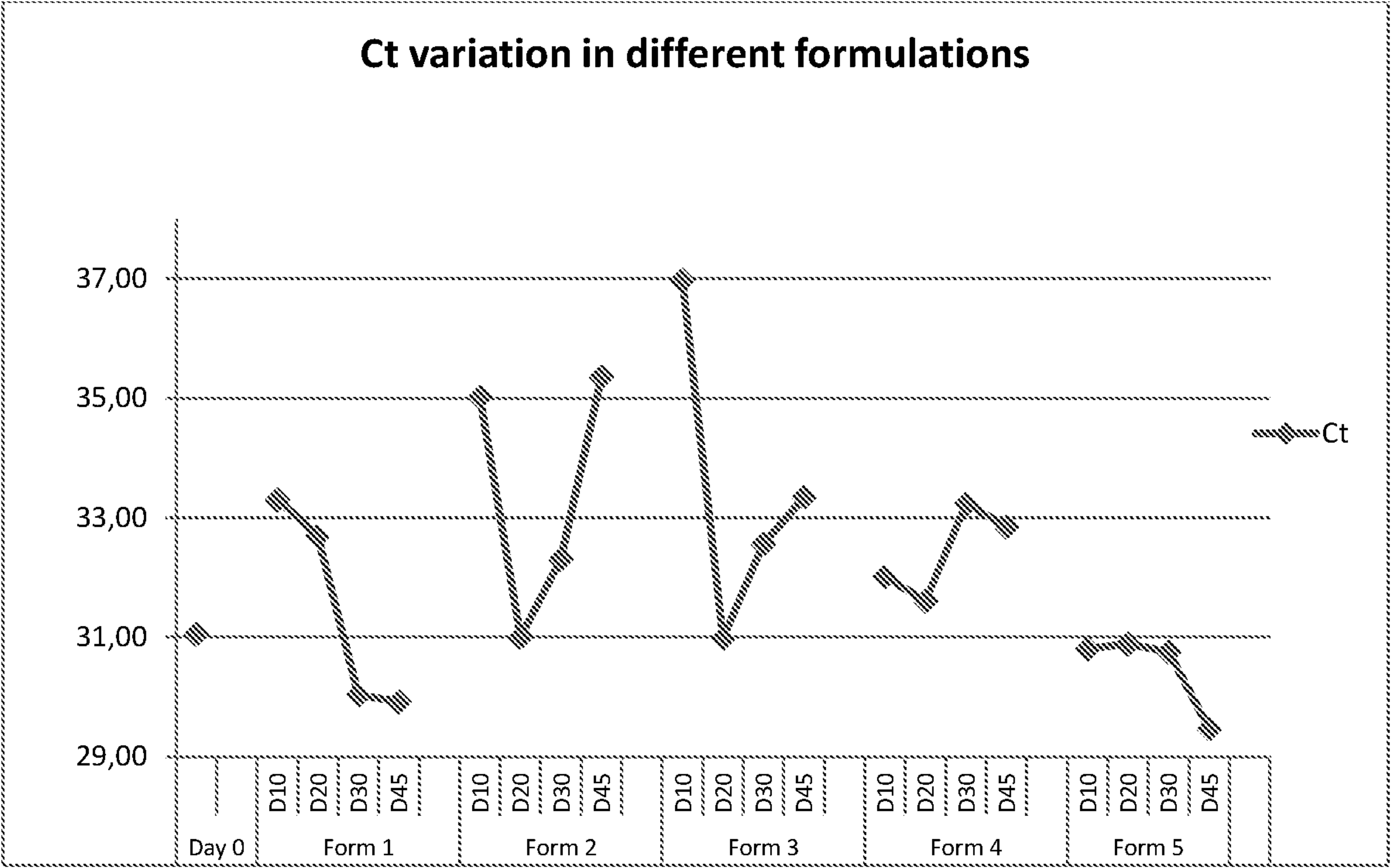


Figure 11



**Figure 12**

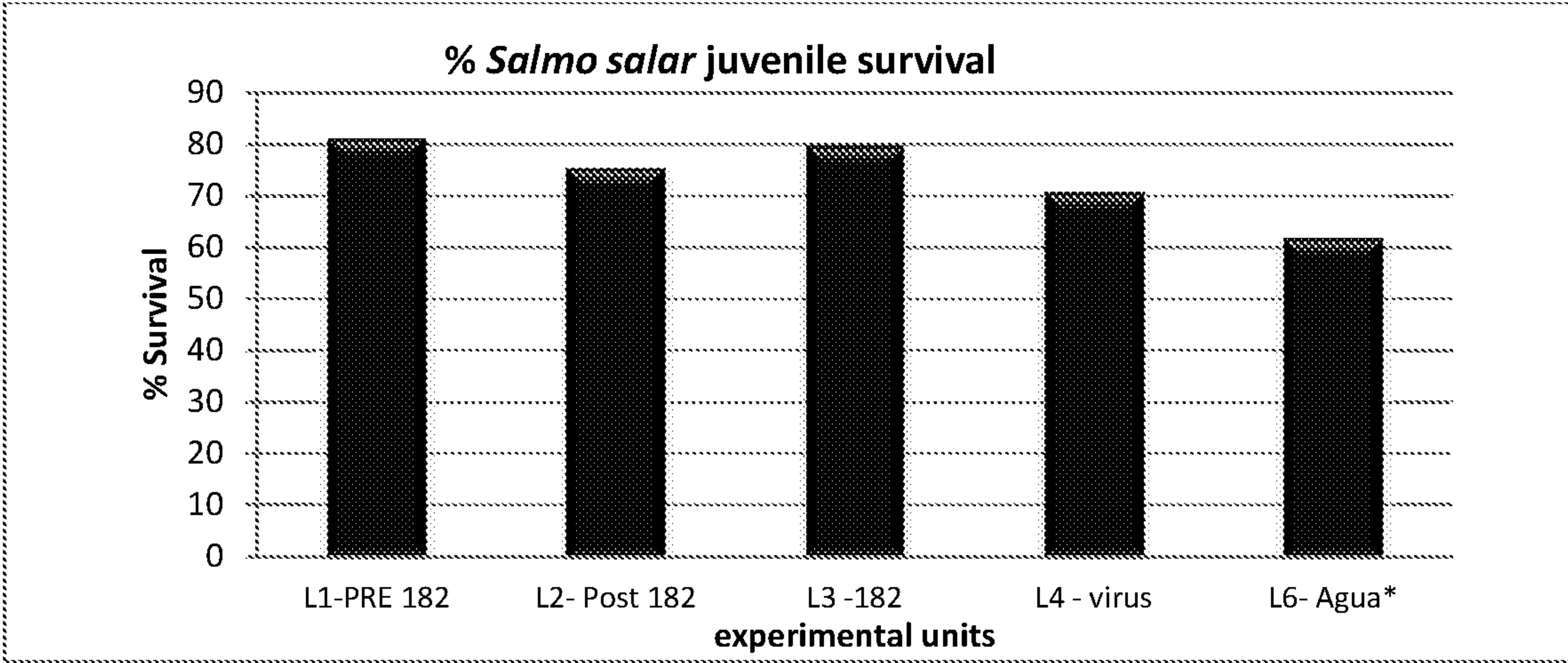


Figure 9

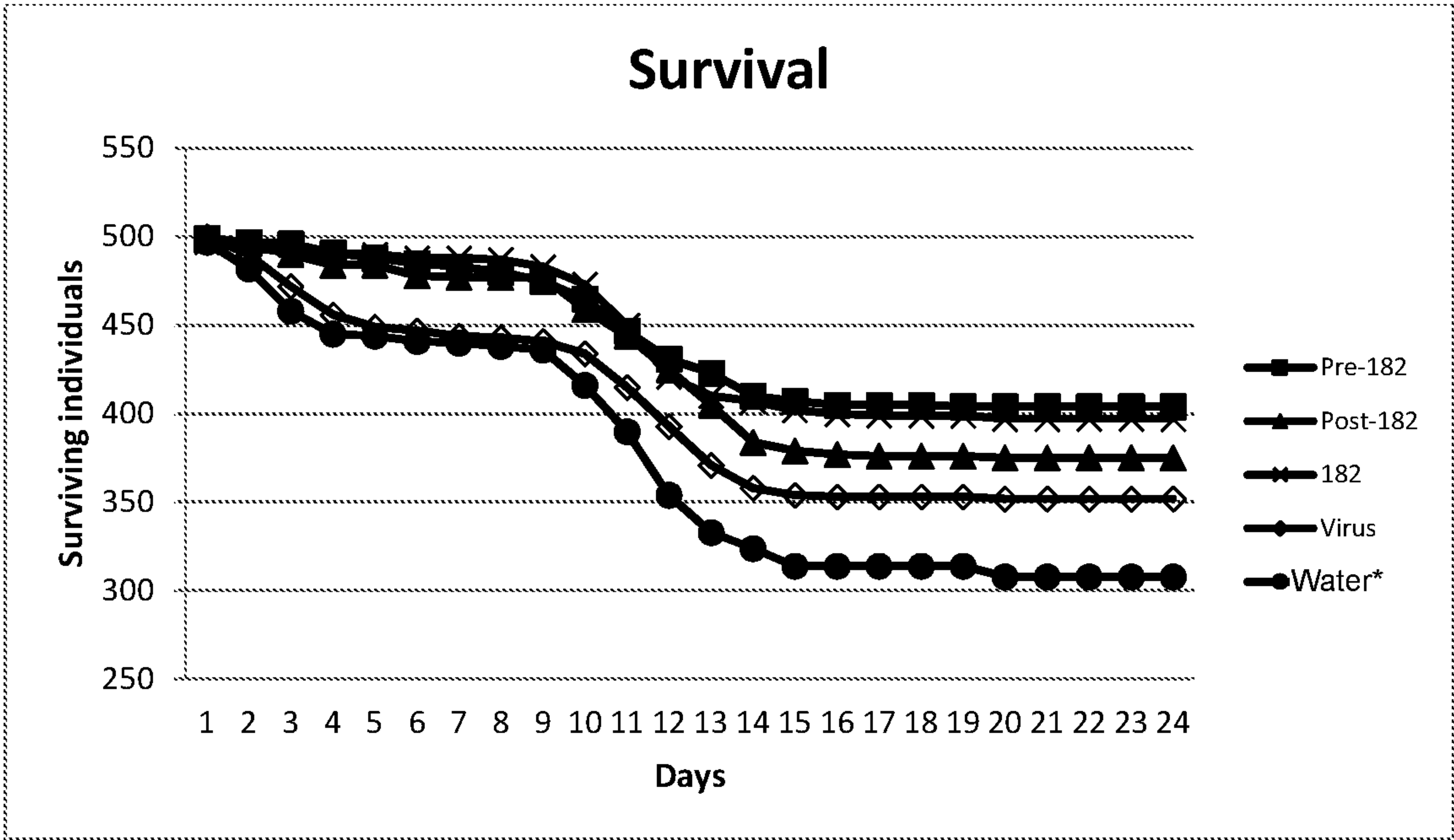


Figure 10