

FISH NODAVIRUS

Global distribution

VNN (Viral nervous necrosis) has been reported from all continents and nodavirus is known to affect a large variety of marine fish worldwide.

Agent

Nodavirus is the aetiological agent of VNN, also called VER (viral encephalopathy and retinopathy). Betanodavirus are small, spherical, non-enveloped viruses with a positive-sense RNA molecule.

Betanodavirus have been classified based on the study of the variable region of the coat protein gene into 4 groups: striped jack nervous necrosis virus (SJNNV), barfin flounder nervous necrosis virus (BFNNV), tiger puffer nervous necrosis virus (TPNNV) and red-spotted grouper nervous necrosis virus (RGNNV).

Pathology

Transmission can occur both horizontally or vertically (for some species) as shown by the detection of virus in the gonads of brood fish, eggs and larvae. Initial introduction of viruses may also come via influent contaminated water.

Clinical signs

This virus is responsible of neurological disorders in an increasing number of marine fish species all over the world. Larvae and juveniles are strongly affected and display abnormal swimming behaviour, high mortality levels and vacuolated lesions in the central nervous system.

Diagnosis

Diagnosis was mainly based on virus isolation on susceptible cell lines followed by virus identification using IFAT (immunofluorescence antibody test). Other techniques such as ELISA or immunochemistry were also available. In the last decade, PCR has offered the possibility of improving sensitivity and rapidity for the diagnosis of nodavirus.

Nodavirus diagnosis in FINALAB

The French national institutes AFSSA and IFREMER were involved in nodavirus detection using PCR and ELISA.

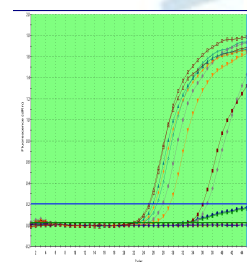
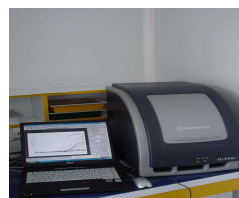
In 2005, nodavirus diagnosis by PCR and ELISA moved from the state institutes to the laboratories LABOFARM and TREGOBIO, two laboratories of the FINALAB Company.

Some additional improvements were added to make the tests useful for our customers:

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An outstanding ELISA test for the routine serological control of Sea bass brood stocks

Dr O JAGOREL, Head manager of TREGOBIO

“The ELISA technique was assigned to our lab from AFSSA institute, the French reference laboratory for nodaviruses. The cut-off position of the test was then established to 0.1OD. This level had been evaluated following analysis of sera coming from fishes maintained under experimental conditions.

However, when the ELISA test was performed on conventional Sea bass sera collected from nodavirus free farms, false positive results were observed.

In collaboration with Dr A LE BRETON, a French aquaculture practitioner, we initiated the follow up of Sea bass brood stocks originating from nodavirus free hatcheries as demonstrated by cell culture and PCR analysis as well as the absence of clinical signs for the past 10 years. Our results have shown that the OD cut-off was underestimated and we decided to set it at 0.5 OD (Table 1) (corresponding to a 1/32 dilution of the AFSSA positive reference serum). The results obtained from the field samples were then in accordance to those that should be obtained from nodavirus free brood stocks. Sera from nodavirus negative and positive Sea bass farms need now to be tested by ELISA to validate definitely this new OD cut-off.”

Effect of changing the cut-off position of Nodavirus Sea bass ELISA test made on nodavirus free fishes, on the breakdown of the population according to negative, doubtful and positive groups.

Nb of sera	AFSSA Interpretation		TREGOBIO new interpretation		
	<0.1 DO: Negative >0.1 DO: Positive		< 0.4 DO: Negative > 0.5 DO: Positive 0.4 < <0.5: doubtful		
	Negative	Positive	Negative	Doubtful	Positive
254	175	79	244	3	7
%	68.9	31.1	96.1	1.2 *	2.7 *

Average value within the population = 0.100 ; Standart deviation = 0.128

* : These values are usually observed when using ELISA commercial kits on non infected populations in animal intensive productions (poultry, swine...)

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Nodavirus PCR test: quality result is closely linked to sample preservation

Many PCR tests have been published for VNN diagnosis. Most of them are based on part of RNA2 detection following reverse transcription and PCR. The specificity of our test was of particular interest: many analysis have been made to ensure that the PCR test was able to detect a wide variety of virus strains including SJNNV and RGNNV. The sensitivity was also our main preoccupation in that faint quantities of virus must be detected by the test.

PCR has now been used for 3 years in the control of brood stock and progeny in France as well as in Mediterranean sea fish producer countries.

Dr PY MOALIC, Technical manager of LABOFARM

“Our customers are expecting rapid, specific and sensitive results when they use PCR, and this is the case with our multispecies nodavirus PCR test. Nevertheless, our experience in the PCR diagnosis area has shown that the reliability of the result is strongly linked to the quality of the sample. In my lab, we receive samples not only from France but also from different European fish producer countries. For this reason, we decided to propose free sampling kits to our customers (producers or veterinarians). These kits are made of specific buffer allowing good conservation of the sample (eyes, brain...) between the sampling area and LABOFARM, at ambient temperature or refrigerated at +10°C. These kits are available on request from our laboratory.”

Positive effect of LABOFARM Nodavirus PCR sampling kit on the results following room temperature conservation

Days after sampling (room temperature)	0	1	4	7
Nodavirus PCR results	Positive	Positive	Positive	Positive

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