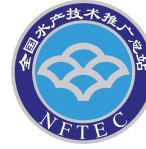




Food and Agriculture
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Yellow Sea Fisheries
Research Institute



National Fisheries Technology
Extension Station



Laboratory Work: IMNV diagnostics field and laboratory protocols (PCR, histopathology)

FAO Project TCP/INT/3501:

Strengthening biosecurity governance and capacities for
dealing with the serious shrimp infectious myonecrosis virus (IMNV)

Final Workshop

Qingdao, China

2-5 November 2017



OIE Reference Laborator
for White spot disease



OIE Reference Laborator
for Infectious hypodermal and haematopoietic necrosis

Asian Regional Laboratory Testing Program For Aquatic Animal Diseases 2013-2014

Manual of Diagnostic Tests for Aquatic Animals

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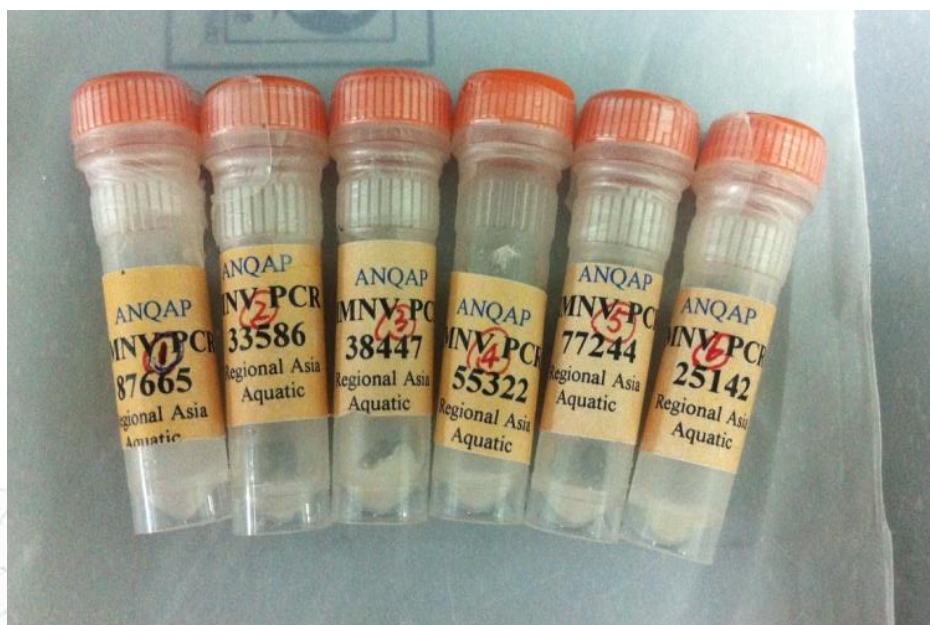
PDF

CHAPTER 2.2.5.

INFECTION WITH INFECTIOUS MYONECROSIS VIRUS

Primer sequences:

Primer	Sequence (5' to 3')	Amplicon Length	Ref.
4587F	CGA-CGC-TGC-TAA-CCA-TAC-AA	328 bp	Poulos & Lightner, 2006
4914R	ACT-CGG-CTG-TTC-GAT-CAA-GT		
4725 NF	GGC-ACA-TGC-TCA-GAG-ACA	139 bp	
4863 NR	AGC-GCT-GAG-TCC-AGT-CTT-G		



Infectious Myonecrosis Virus (IMNV) PCR Proficiency Testing Report

Testing date:	Round 3, June 2014
Report date:	7 th July 2014
Number of laboratories participating:	18
Number of laboratories passed:	15

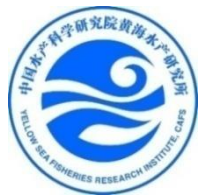
Table 2: Summary of Round 3 PCR results for IMNV.

Laboratory No.	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Round 3 Results	R3 Score	R2 Score	R1 Score
Expected Interpretation	Neg	Neg	Low Pos	High Pos	Moderate Pos	Moderate Pos				
34	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	6	2
36	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	-	-
37	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	4	5
38	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	6	6
39	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	6	4
42	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	5	2
48	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	2	2
49	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	6	6
54	Neg	Neg	Pos (Ct 32.50)	Pos (Ct 23.57)	Pos (Ct 28.85)	Pos (Ct 29.29)	PASS	6	6	5
55	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	6	6
56	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	6	-
57	Neg (Ct >40)	Neg (Ct >40)	Pos (Ct 25.60)	Pos (Ct 23.04)	Pos (Ct 27.61)	Pos (Ct 23.53)	PASS	6	6	6
58	Neg	Neg	Neg	Pos	Pos	Pos	V	5	6	5
63	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	6	5
64	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	-	-
66	Neg	Neg	Neg	Pos	Pos	Pos	V	5	5	2
67	Neg	Neg	Neg	Pos	Pos	Pos	V	5	-	-
68	Neg	Neg	Pos	Pos	Pos	Pos	PASS	6	6	6
Expected Interpretation	Neg	Moderate Pos	Low Pos	High Pos	Moderate Pos	Moderate Pos				

PASS All results for test round correct



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Sources of samples:

1. Positive control came from Indonesia, IMNV infected *P.vannamei*
2. Negative control came from USA, SPF *P.vannamei*



Step1: Sample preprocessing

中国水产科学研究院黄海水产研究所水生动物疫病诊疗中心 CAADDC-TR-01

检测委托单

编号:

客户联系信息

客户名称: 地址:

联系人: 电话:

客户其它要求

报告格式: 中文 英文 中英文

特殊保密: 需要 不需要

报告送达: 邮寄 传真 自取

要求完成时间:

送样须知 1、送样人对样品的代表性、真实性负责; 检测单位的检测结果仅对送样人送检样品负责; 2、送样人未填写检测标准/方法, 视为同意采用检测单位所选用的检测标准/方法;
3、送检样品须满足检测单位的检验要求; 验余样品如需返还, 请事先声明; 4、本检测委托单一式二联, 第一联由检测单位保存; 第二联交客户保存。

序号	客户标识	品种	状态	规格/数量	检测项目	检测标准/方法
1			<input type="checkbox"/> 活体 <input type="checkbox"/> 冷冻 <input type="checkbox"/> 新鲜 <input type="checkbox"/> E95 <input type="checkbox"/> 其它		<input type="checkbox"/> WSSV <input type="checkbox"/> IHNV <input type="checkbox"/> 其它:	WSSV: <input type="checkbox"/> GB/T 28630.2-2012 <input type="checkbox"/> OIE 手册(现行) IHNV: <input type="checkbox"/> GB/T 25878-2010 <input type="checkbox"/> OIE 手册(现行) 其它:
2			<input type="checkbox"/> 活体 <input type="checkbox"/> 冷冻 <input type="checkbox"/> 新鲜 <input type="checkbox"/> E95 <input type="checkbox"/> 其它			
3			<input type="checkbox"/> 活体 <input type="checkbox"/> 冷冻 <input type="checkbox"/> 新鲜 <input type="checkbox"/> E95 <input type="checkbox"/> 其它			
4			<input type="checkbox"/> 活体 <input type="checkbox"/> 冷冻 <input type="checkbox"/> 新鲜 <input type="checkbox"/> E95 <input type="checkbox"/> 其它			
5			<input type="checkbox"/> 活体 <input type="checkbox"/> 冷冻 <input type="checkbox"/> 新鲜 <input type="checkbox"/> E95 <input type="checkbox"/> 其它			
6			<input type="checkbox"/> 活体 <input type="checkbox"/> 冷冻 <input type="checkbox"/> 新鲜 <input type="checkbox"/> E95 <input type="checkbox"/> 其它			
7			<input type="checkbox"/> 活体 <input type="checkbox"/> 冷冻 <input type="checkbox"/> 新鲜 <input type="checkbox"/> E95 <input type="checkbox"/> 其它			
8			<input type="checkbox"/> 活体 <input type="checkbox"/> 冷冻 <input type="checkbox"/> 新鲜 <input type="checkbox"/> E95 <input type="checkbox"/> 其它			
9			<input type="checkbox"/> 活体 <input type="checkbox"/> 冷冻 <input type="checkbox"/> 新鲜 <input type="checkbox"/> E95 <input type="checkbox"/> 其它			
10			<input type="checkbox"/> 活体 <input type="checkbox"/> 冷冻 <input type="checkbox"/> 新鲜 <input type="checkbox"/> E95 <input type="checkbox"/> 其它			

送样人(签字): 年月日

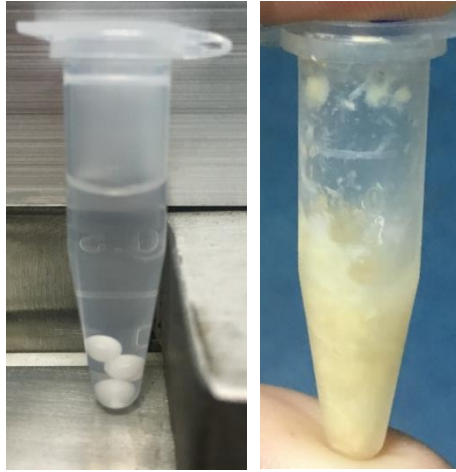
业务收发员(签字):

年月日

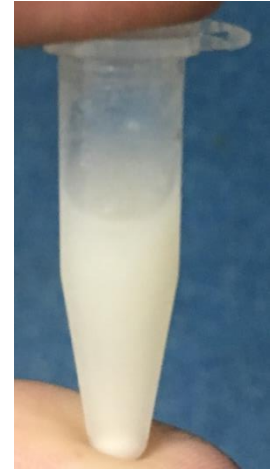
地址: 山东省青岛市南京路106号 266071 电话: 0532-85823062 传真: 0532-85811514 Email: oie@ysfri.ac.cn

第一联: 本单位保存

Before



After



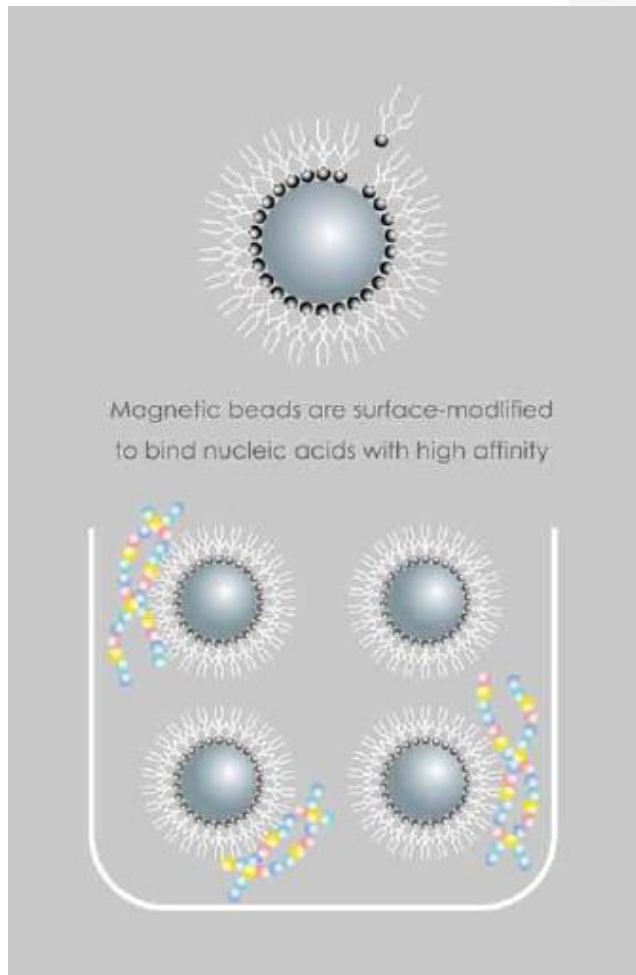
Homogenate





Step2: *Extract nucleic acid*

Nucleic Acid Automatic Extraction System

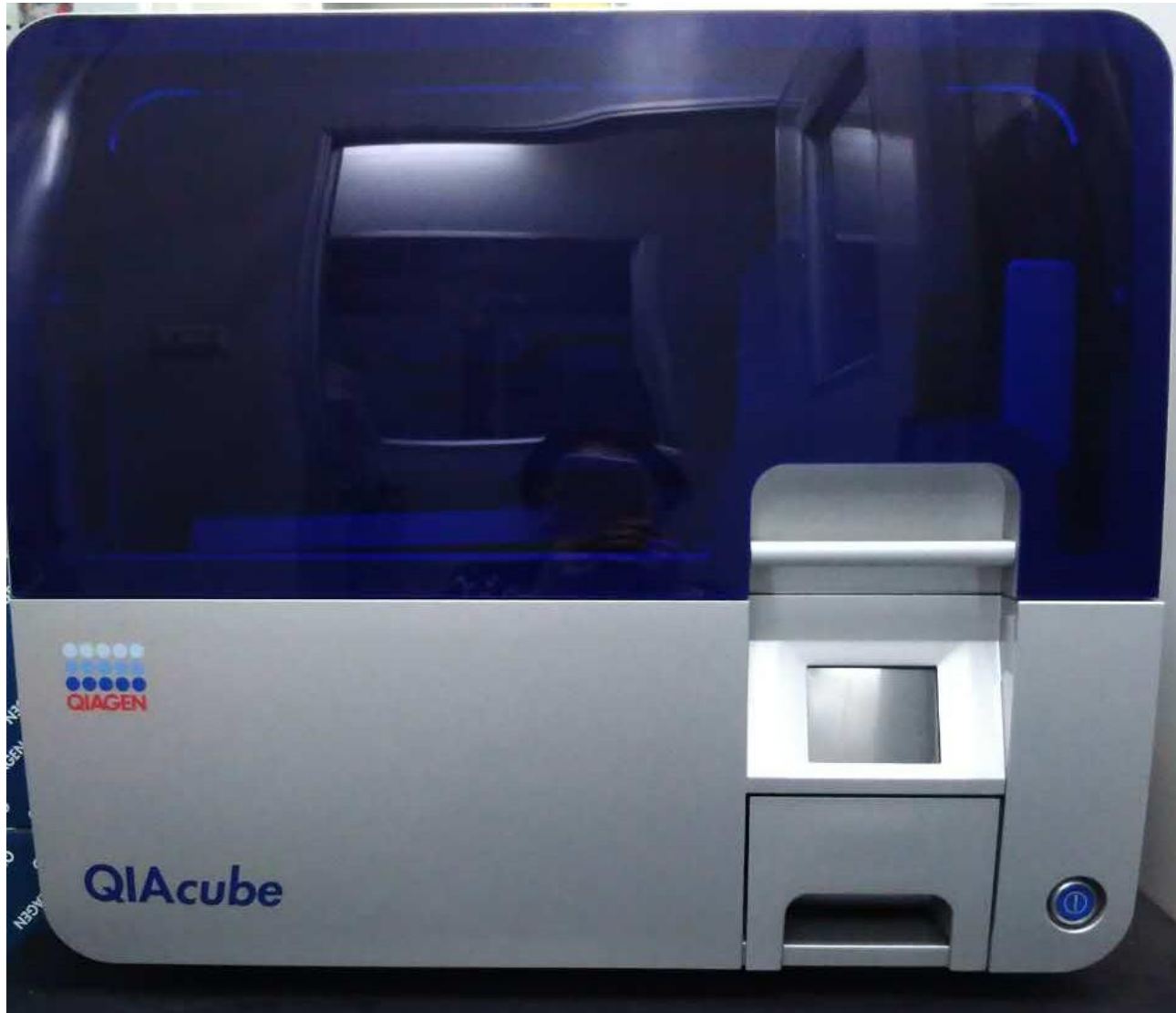


taco™ mini 自动核酸提取仪



Step2: *Extract nucleic acid*

Nucleic Acid Automatic Extraction System





Step 3: *Molecular techniques*

qRT-PCR

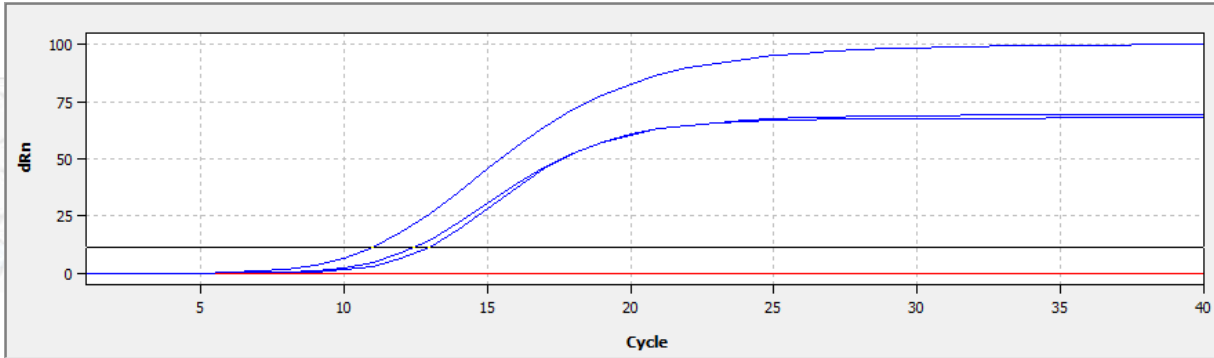
Analytik Jena





Step3: Molecular techniques

qRT-PCR



qPCRsoft 3.2 - [Real time pcr project - 20171104-IMNV.rtp]

ddCt Quantification: 1

Gene of Interest (GOI): FAM Group: 组 1 Threshold: 11.526

Reference genes: FAM

Well	Sample name	Sample type	Group	Ct GOI	Mean Ct GOI	Mean Ct R...	Std.Dev. ...	dCt GOI	dCt Ref.g...	RQ GOI
D3	N1	Negative control	组 1	No Ct						
F3	N1	Negative control	组 1	No Ct						
B8	N1	Negative control	组 1	No Ct						
D4	P1	Positive control	组 1	13.01	12.74	13.21	0.39	-11.74	-12.21	0
F4	P1	Negative control	组 1	11.01	11.01	11.49	0	-10.01	-10.49	0
B9	P1	Positive control	组 1	12.46	12.74	13.21	0.39	-11.74	-12.21	0

Table | Standard curve | Validation |



Thank you

