## Accepted Manuscript

The development and application of a duplex reverse transcription loop-mediated isothermal amplification assay combined with a lateral flow dipstick method for *Macrobrachium rosenbergii* nodavirus and extra small virus isolated in China

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## ACCEPTED MANUSCRIPT

1	The development and application of a duplex reverse transcription
2	loop-mediated isothermal amplification assay combined with a
3	lateral flow dipstick method for Macrobrachium rosenbergii
4	nodavirus and extra small virus isolated in China
5	
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15	
16	Abstract: White tail disease (WTD), a major disease prevailing in the larval stage of
17	Macrobrachium rosenbergii, caused by Macrobrachium rosenbergii nodavirus
18	(MrNV) associated with extra small virus (XSV), led to the economic loss of shrimp
19	industry in China. In order to establish a convenient, sensitive and selective molecular
20	diagnostic method to detect MrNV and XSV for the Chinese shrimp
21	(MrNV/XSV-chin), a reverse transcription loop-mediated isothermal amplification
22	(RT-LAMP) assay combined with a lateral flow dipstick (LFD) method were
23	developed. A set of four specific primers and a labeled probe were designed according
24	to the six conserved gene sequence regions encoding for the MrNV capsid protein
25	CP43 and the XSV capsid protein CP17. The detection of MrNV and XSV
26	simultaneously by RT-LAMP was performed at 61°C in a single reaction for 60 min
27	followed by hybridization with an FITC-labeled probe for 5 min and visualized by
28	LFD. The RT-LAMP-LFD assay had a sensitivity of approximately 100-fold higher
29	than conventional PCR. In addition, the assay could detect MrNV/XSV-chin from
30	limited amount of RNA extracts as low as 1.0 pg extracted from Macrobrachium
31	rosenbergii. This assay was simple to use, required little instrumentation, and
32	exhibited excellent specificity for the MrNV/XSV-chin compared with other shrimp
33	viruses. In conclusion, a convenient, sensitive and selective practical molecular
34	diagnostic method was developed with the potential for diagnosis and prevention of
35	WID.
36	<b>Key words:</b> <i>Macrobrachium rosenbergu</i> nodavirus; extra small virus; duplex Reverse
3/ 20	iranscription; loop-mediated isotnermal amplification; lateral flow dipstick
38 20	
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## **1. Introduction**

*Macrobrachium rosenbergii* is an important economic crustacean that is farmed in

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