

4.A1.B.7 Worksheet B.7 – Viral Hemorrhagic Septicemia Virus (VHSV)

Case Number _____

Date _____

Master Mix

PCR Reagent	Lot #	Final Concentration	Stock Concentration	Volume per Reaction (µL) (to total 50 µL)	Volume for _____ samples
d-H ₂ O*		-	-	23.75 µL	
PCR buffer (no MgCl ₂)		1X	10X	5 µL	
MgCl ₂		2.5 mM	25 mM	5 µL	
dNTP's		0.2 mM	2 mM	5 µL	
AMV reverse transcriptase		4.5 Units/Rx	9 Units/µL	0.5 µL	
(+)Primer		50 pmoles/Rx	20 pmoles/µL	2.5 µL	
(-)Primer		50 pmoles/Rx	20 pmoles/µL	2.5 µL	
TAQ		2.5 Units/Rx	5 Units/µL	0.5 µL	
RNasin		9.75 Units/Rx	39 Units/µL	0.25 µL	
RNA template		-	-	5 µL	-

*Add nuclease free water to Master Mix first, TAQ last.

Primer Sets for VHSV

Forward	Reverse
5'-GGG-GAC-CCC-AGA-CTG-T-3'	5'-TCT-CTG-TCA-CCT-TGA-TCC-3'

Control Information

POSITIVE CONTROLS		NEGATIVE CONTROLS	
Extraction	PCR	Extraction	PCR

Amplification (Thermocycle Process)

Date & Time	Program #	NOTES

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Gel Preparation

Gel Concentration	Apparatus and Gel Size (Mini = 8-22 wells; Midi = 22-40)	Weight of Agarose (grams)	Volume of Buffer (mL)

Gel Template (Sample Placement Map)

Ladder Brand / Lot # _____ Loading Buffer Brand / Lot # _____

Enter sample ID below corresponding well number:

PCR Products (Loaded LEFT to RIGHT)														
<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>14</u>	<u>15</u>
<u>16</u>	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>	<u>25</u>	<u>26</u>	<u>27</u>	<u>28</u>	<u>29</u>	<u>30</u>