Infectious Bovine Rhinotracheitis Viral gG Expression and gG-ELISA Development

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Abstract Taking the genome DNA of Infectious Bovine Rhinotracheitis Virus [IBRV] as the template, the gG gene was ampliﬁed with PCR and cloned into the T cloning vector pMD18-T. After being identiﬁed by restriction digestion and DNA sequencing, the insert was subcloned into the expression vector pGEX-KG. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] and Western blot assay showed that this gene was expressed as both soluble form and inclusion body by the transformed E. coli BL21 strain DE3. The fusion protein was puriﬁed and used as the coating antigen to develop the indirect Enzyme-Linked Immunosorbent Assay [ELISA]. Comparison between this gG-ELISA and commercial IBRV gB-ELISA 

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Ki67 IDEXX was made in the detection of 380 cow serum samples. The results demonstrated an agreement of 92%. By using this novel gG-ELISA on 248 cow serum samples were tested and the average positive rate of IBRV antibodies for imported cows is 21.7% while the positive rate ranged greatly from 0.0% ~ 41.5% for Hubei local Chinese Black and White Dairy Cows.

**Key words** Infectious Bovine Rhinotracheitis Virus gG gene ELISA prokaryotic expression

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1

1.1

1.1.1 Bartha

1.1.2 pMD18-T Taq

1.1.3 E. coli DH5α

1.1.4 IDEXX

1.2

1.2.1 IBRV Bartha Nu/67

1.2.2 MDBK 48 ~ 72h 80%

1.2.3 Optima™ LE-80K Ultracentrifuge SW28 rotor 27000 r/min × 120min TEN 100.0 mmol/L NaCl 10.0 mmol/L Tris-HCl 1.0 mmol/L EDTA pH 8.0

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颜邦芬等:牛传染性鼻气管炎病毒 gG 蛋白的表达及 gG-ELISA 的建立

1.2.2 长核苷酸序列
27000r/min × 120min, 2% (NH₄)₂SO₄, 2% EDTA, 100μg/ml, KCl
37℃, 30min
75% DNA, 20℃ - 20℃, 20℃, 20℃.

1.2.3 GenBank Bartha Nu/67 gG: NC001847

1.2.4 gG: PCR 50μL, DNA 1μL, dNTP 2μL, DMSO 5μL, 10× Buffer 5μL, Taq 1μL

1.2.5 gG: PCR pMD18-T

1.2.6 Western blot BL21 DE3

1.2.7 ELISA IBRV gG: IgG

2

2.1

2.2 PCR

IBRV Bartha Nu/67 DNA
gG: 1.3kb, 1.3kb pMD18-T, BamH I

IBRV

IBRV
**2.3 gG-ELISA**

```plaintext
0.8mmol/L IPTG 3h Western blot SDS-PAGE

Glutathione 4B pGEX-gG 73kDa Western blot

pGEX-KG 1335bp [GenBank: NC001847] ATG TGA

GenBank: IBRV Bartha Tu

GenBank: gG ELISA BL2 DE3

Fig. 1 The expression of gG recombinant protein analyzed with SDS-PAGE and Western blot

Table 1 The results of repetition test for gG-ELISA

| In-batch CV/% | 8.8 | 3.5 | 5.9 | 8.6 | 6.7 | 7.1 |
| Between batch CV/% | 9.1 | 4.5 | 6.2 | 7.3 | 7.0 | 5.4 |

Table 2 The results of specificity test for gG-ELISA
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**2.4.1 ELISA**

```plaintext
1 2 3 4 5 6

1 SDS-PAGE Western blot gG ELISA

Fig. 1 The expression of gG recombinant protein analyzed with SDS-PAGE and Western blot
```

**2.4 gG-ELISA**

<table>
<thead>
<tr>
<th>Diseases</th>
<th>IBR</th>
<th>FMD</th>
<th>BVD</th>
<th>BT</th>
<th>BB</th>
<th>BED</th>
<th>Br</th>
<th>IBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD&lt;60</td>
<td>0.112</td>
<td>0.149</td>
<td>0.137</td>
<td>0.203</td>
<td>0.117</td>
<td>0.185</td>
<td>0.26</td>
<td>0.511</td>
</tr>
<tr>
<td>OD&gt;60</td>
<td>0.299</td>
<td>0.299</td>
<td>0.299</td>
<td>0.299</td>
<td>0.299</td>
<td>0.299</td>
<td>0.299</td>
<td>0.299</td>
</tr>
</tbody>
</table>

**2.4.2 pGEX-KG**

10min 60 10min OD<60 X ± 0.025

**2.4.3 pGEX-KG**

3.0% 1.0% 1.5% 2.0% 2.5%

**2.4.4 pGEX-KG**

1.0% 1.0% 1.0% 1.0% 1.0% 1.0%

**2.4.5 pGEX-KG**

1.0% 1.0% 1.0% 1.0% 1.0% 1.0%

<table>
<thead>
<tr>
<th>Diseases</th>
<th>IDEXX</th>
<th>IBRV</th>
<th>gB-ELISA</th>
<th>gG-ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD&lt;60</td>
<td>0.26%</td>
<td>20%</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>OD&gt;60</td>
<td>83%</td>
<td>60%</td>
<td>72%</td>
<td>72%</td>
</tr>
</tbody>
</table>

**2.4.6 pGEX-KG**

18.95% 18.95% 18.95% 18.95% 18.95% 18.95%
表3 比较gG-ELISA和商业gB-ELISA IDEXX

<table>
<thead>
<tr>
<th>批号</th>
<th>商品gB-ELISA</th>
<th>gG-ELISA</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>291</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>308</td>
</tr>
<tr>
<td>4</td>
<td>77</td>
<td>0.443 ± 0.115</td>
</tr>
<tr>
<td>5</td>
<td>303</td>
<td>0.151 ± 0.068</td>
</tr>
<tr>
<td>6</td>
<td>0.077 ± 0.062</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.996 ± 0.158</td>
<td></td>
</tr>
</tbody>
</table>

表5 牛群血清抗体检测

<table>
<thead>
<tr>
<th>组别</th>
<th>总头数</th>
<th>阳性头数</th>
<th>阳性率%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>441</td>
<td>121</td>
<td>27.4</td>
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<tr>
<td>2</td>
<td>63</td>
<td>5</td>
<td>7.9</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
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<td>0.0</td>
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<td>4</td>
<td>129</td>
<td>12</td>
<td>9.3</td>
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<td>5</td>
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<td>5</td>
<td>14.7</td>
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<tr>
<td>6</td>
<td>65</td>
<td>27</td>
<td>41.5</td>
</tr>
<tr>
<td>7</td>
<td>483</td>
<td>105</td>
<td>21.7</td>
</tr>
<tr>
<td>总计</td>
<td>1248</td>
<td>275</td>
<td>22.0</td>
</tr>
</tbody>
</table>

*Impruned cows.*

2.4.6 gG-ELISA与gB-ELISA IDEXX的比较试验

与商品试剂盒的比较试验，发现两种方法具有较高的符合率，证实所建立的方法可用于临床检测。
REFERENCES


