Isolation of *Vibrio vulnificus* from Oyster Homogenate by Immunomagnetic Separation Using Anti-H Monoclonal Antibodies

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**ABSTRACT:** *Vibrio vulnificus* is cited as a sole cause of about 350 illnesses per year in the United States with an overall 40% mortality rate. Conventional methods for the detection and enumeration of *V. vulnificus* from environmental samples are labor-intensive and time-consuming. Immunomagnetic beads (IMB) coated with monoclonal antibody specific for *V. vulnificus* could prove useful for the isolation and concentration of the organism from complex environmental samples. This study was aimed at developing an immunomagnetic separation protocol using anti-H monoclonal antibody for the recovery of *V. vulnificus* from spiked oyster homogenates. Two different sets of IMB were prepared by mixing sheep anti-mouse IgG IMB with monoclonal antibodies reactive with *V. vulnificus* flagellar core at a concentration of 5 µg IgG/10^7 IMB. The binding capacity of the beads coated with monoclonal antibodies was determined by incubating 10^7 IMB with 500 µl spiked oyster homogenate on a shaker at 25°C for 30 minutes followed by separation with an immunomagnetic bead concentrator. The number of unbound bacteria was determined by plating the aspirated supernatant fluid on TCBS and TSA plus 2% NaCl agar plates. Two clinical strains of *V. vulnificus* at two different concentrations, 1-3X10^8 and 1-3 X10^9 *V. vulnificus/ml* respectively, exhibited binding capacities up to 35 and 62% dependent on cell concentration and monoclonal antibody employed. This technique could lead to a simple and rapid method for oyster risk assessment.

**DISCUSSION:**

- At 10^7 CFU/ml mean percentage binding of 1007 with MAB 8-D-4 was determined to be the highest at 57%, followed by CT164 at 52%. Binding of ATCC 27562 was 35% which is significantly lower than the other two strains. At 10^2 CFU/ml the binding percentage for three strains was about 25%.
- MAB 3-D-10 at 10^2 CFU/ml showed the highest percentage mean binding of 50% in 1007. The binding of ATCC 27562 was 29%, and ATCC 27562 and 1007 were 29%, 26 and 25% respectively.
- Difference between the percentage binding of ATCC 27562, 1007 and CT164 might be due to nonmotile cells present in the ATCC strain.

**CONCLUSIONS:**

MAB could be used in an IMS method to concentrate and detect *V. vulnificus* from mixed cultures, seawater and shellfish homogenate.

**REFERENCES:**


**ACKNOWLEDGEMENTS:**

This research was funded by Louisiana Sea Grant College Program.

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**METHOD:**

**IMMUNOMAGNETIC SEPARATION (IMS):**

1. 10 g of Oyster meat
2. 20 ml of Phosphate buffer saline
3. Homogenization followed by filtration through cheese cloth
4. Filtrate spiked with *Vibrio vulnificus* to reach 10^8 or 10^9 CFU/ml
5. 500 µl of spiked oyster homogenate
6. 10^7 immunomagnetic beads coated with 5 µg MAB 8-D-4 or MAB 3-D-10
7. 30 min incubation on shaker
8. IMB removed and fluid diluted and plated to determine *V. vulnificus* CFU/ml

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**RESULTS:**

**IMMUNOMAGNETIC SEPARATION (IMS):**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Strain</th>
<th>ATCC</th>
<th>Clinical 1007</th>
<th>Clinical CT164</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-D-4</td>
<td>Vv 10^8 CFU/ml</td>
<td>59.32%</td>
<td>58.12%</td>
<td>56.80%</td>
</tr>
<tr>
<td>3-D-10</td>
<td>Vv 10^5 CFU/ml</td>
<td>69.02%</td>
<td>67.32%</td>
<td>65.92%</td>
</tr>
<tr>
<td>3-D-10</td>
<td>Vv 10^4 CFU/ml</td>
<td>61.52%</td>
<td>59.78%</td>
<td>58.06%</td>
</tr>
<tr>
<td>3-D-10</td>
<td>Vv 10^3 CFU/ml</td>
<td>61.57%</td>
<td>59.62%</td>
<td>58.22%</td>
</tr>
</tbody>
</table>

**DISCUSSION:**

- Table 1: Percentage binding of IMS with different *V. vulnificus* strains at various concentrations

**CONCLUSIONS:**

- MAB could be used in an IMS method to concentrate and detect *V. vulnificus* from mixed cultures, seawater and shellfish homogenate.