**Isolation of *Vibrio vulnificus* by Immunomagnetic Separation using Anti-H Monoclonal Antibodies**

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**ABSTRACT:** *Vibrio vulnificus* is considered one of the most lethal of all human pathogenic vibrios, accounting for 95% of all seafood related deaths in the United States. One approach to improve the recovery and detection sensitivity of *V. vulnificus* without sacrificing assay time is through the use of immunomagnetic separation (IMS). The aim of this study was to develop and optimize an IMS protocol using anti-H antibody for the concentration of *V. vulnificus* from phosphate buffered saline (PBS) suspensions. Six monoclonal antibodies were produced by immunizing mice at 2 week intervals by injection of 50 µg of purified *V. vulnificus* ATCC 27562 flagellin. Murine spleen cells were collected and fused with myeloma cells for hybridoma production. Antibodies were purified from cloned hybridomas secreting anti-H IgG and analyzed by ELISA using *V. vulnificus* polar flagellar cores bound to microtiter plates. Antibody exhibiting a high anti-H titer was then coated onto sheep anti-mouse IgG immunomagnetic beads (IMB) at a concentration of 4µg/10⁷ beads. The antibody-coated IMB were mixed with *Vibrio* cell suspensions on slides and coagglutination reactions observed. To determine binding capacity, 10⁷ IMB were coated with 5 µg monoclonal IgG and incubated with 10³ *V. vulnificus* cells/ml PBS on a shaker at 25°C for 30 min. After magnetic separation, the aspirated supernatant fluid was spread onto agar plates, incubated overnight and the number of unbound *V. vulnificus* cells determined.

**INTRODUCTION:** In the United States, seafood accounts for over 26% of food-borne disease outbreaks due to consumption of raw or undercooked molluscan shellfish contaminated with vibrios. Due to the severity of *Vibrio vulnificus* infection, Gulf Coast oysters are under intense scrutiny. There is a need to develop an assay for identification of *V. vulnificus* which is fast, sensitive and specific. One approach is to use a serological test for detection of *V. vulnificus* which would react with species-specific antigens. Monoclonal anti-H antibodies (MAB) were produced to provide a continual source of specific antibody for use in *V. vulnificus* immunosassays and their potential use in an IMS protocol was examined.

**METHODS:**

- **MAB PRODUCTION:**
  - Serial dilutions of 16-18 hr *V. vulnificus* broth culture were made in PBS to reach 10⁷ cells/ml.
  - 50 µl bacterial suspension mixed with 20 µl (10⁷) immunomagnetic beads coated with 5 µg MAB (IMB).
  - After 30 min incubation on shaker, IMB were separated with the help of magnetic particle concentrator.
  - Supernatant fluid was serial diluted and plated on nutrient agar.

- **SLIDE COAGGLUTINATION:**
  - Two-fold dilutions with a beginning IgG concentration of 27 µg/well and 1 µg *Vv* flagellin bound/well.
  - *V. vulnificus* isolates were tested serologically against nine different *V. vulnificus* species and 41 *V. parahaemolyticus* and 70 *V. vulnificus* clinical and environmental strains by slide agglutination.

**RESULTS:**

- **IMMUNOMAGNETIC SEPARATION (IMS):**
  - Serial dilutions of 16-18 hr *V. vulnificus* broth culture were made in PBS to reach 10⁷ cells/ml.
  - 50 µl bacterial suspension mixed with 20 µl (10⁷) immunomagnetic beads coated with 5 µg MAB (IMB).
  - After 30 min incubation on shaker, IMB were separated with the help of magnetic particle concentrator.
  - Supernatant fluid was serial diluted and plated on nutrient agar.

- **SLIDE AGGLUTINATION:** Anti H-coagglutination reagents were prepared by mixing 4 µg of MAB with 10³ sheep anti-mouse IgG immunomagnetic beads (Invitrogen). Each of the two anti-H coagglutination reagents were tested serologically against nine different *Vibrio* species and 41 *V. parahaemolyticus* and 70 *V. vulnificus* clinical and environmental strains by slide agglutination.

**CONCLUSIONS:** MAB raised against *V. vulnificus* polar flagella could be used to differentiate *V. vulnificus* from other *Vibrio* species by slide agglutination. MAB could be used in an IMS method to concentrate and detect *V. vulnificus* from mixed cultures, seawater and shellfish homogenate.

**REFERENCES:**

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