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

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*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* determined. Results: Starting with 27 µg IgG, ELISA anti-H titers ranged from 256 to >2048. The antibody-coated IMB coagglutinated 70 (100%) of *V. vulnificus* clinical and environmental strains within 30 sec and did not react with 8 different *Vibrio* spp., including 42 *V. parahaemolyticus* strains. IMB coated with antibody bound between 13-61% of the bacteria at a concentration of 103 *V. vulnificus*/ml.