Monoclonal antibody production: a comparison of *in vitro* and *in vivo* methods and their use in Clostridial vaccine manufacture.

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Abstract

The genus *Clostridium* contains rod-shaped, endospore forming, gram-positive bacteria that are obligate anaerobes (Delano, Mischler, & Underwood, 2002; Hatheway, 1990; Rood, 1997). Clostridial diseases are important diseases of livestock in New Zealand, and are considered by some to be the most economically important diseases of sheep and other livestock (Walker, 1992). They are characterized by systemic vascular failure and/or necrotizing enteritis within hours after exposure—a speed that out-paces a naïve individual's ability to control and effectively counteract the toxin's effects. Vaccination is an important management practice that can decrease the morbidity and mortality associated with Clostridial infections, and vaccination has been used safely and successfully in New Zealand livestock for many years.

Vaccine manufacture and quality assurance (QA) often involves production of monoclonal antibodies (MAbs) derived from culture of hybridoma cells. Traditionally, large numbers of animals have been used to support research, development, and manufacture of Clostridial vaccines (for use in toxin neutralization, vaccine challenge studies, and potency determination) and for MAb manufacture (ascites model). There is currently a great emphasis on finding ways to reduce, refine, and replace animal use in research.

Studies were undertaken that involved MAb production techniques and assay development related to *C. perfringens* Type C beta toxin and Type D epsilon toxin. Two different methods for MAb production were evaluated: a traditional *in vivo* murine ascites method and an *in vitro* method based on use of a commercially available two chamber plastic bioreactor system (CELLine; Becton Dickinson). Two hybridoma lines with historic MAb activity against the lethal and dermo-necrotizing effects of epsilon toxin (EP82) and beta toxin (CP68) were cultured in each production system. In addition to comparing the quantity of MAbs produced by each method, a sandwich ELISA based on use of the anti-epsilon MAbs was developed. This ELISA was implemented for use as a diagnostic tool for internal investigation of batches of epsilon toxoid-containing vaccines that were identified as having low potency in the standard QA test (rabbit antibody induction) for potency.

These studies showed that an *in vitro* method of hybridoma culture was more time and cost effective than conventional live animal ascites production, based on the total quantity of

MAb produced for both of the hybridoma lines that were studied. Additionally, the sandwich ELISA that was developed was effective in detecting very small amounts of toxoid.

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Abbreviations

BA/BSA	Bovine albumin, bovine serum albumin
CCM	Cell compartment media
CDC	United States Centers for Disease Control and Prevention
CMI	Cell-mediated immunity
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
FPLC	Fast protein liquid chromatography
IgG	Immunoglobulin G
MAb	Monoclonal antibody
mAU	Absorbance units
MHC	Major histocompatibility complex
NCM	Nutrient compartment media
PET	Polyethylene terephthalate
PBS	Phosphate buffered saline
OD	Optical density
QA	Quality assurance