Title: **Simultaneous Measurement of Specific Serum IgG Responses to Five Select Agents**

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Select Agents are defined by CDC and the USDA Animal and Plant Health Inspection Service (APHIS) as biological agents or toxins deemed a threat to the public, animal or plant health, or to animal or plant products. They are classified based on their ease of dissemination, mortality/morbidity rate, and potential for social disruption. A subset of these agents includes *Bacillus anthracis, Yersinia pestis, Francisella tularensis*, ricin toxin (RT) and staphylococcal enterotoxin B (SEB). Infection or intoxication with these agents has been shown to elicit antigen specific serum IgG responses. We describe a fluorescent covalent microsphere immunoassay (FCMIA) to measure specific IgG antibodies to seven different antigens from five different select agents: *B. anthracis* (protective antigen [PA] and lethal factor [LF]), *Y. pestis* (F1 and V antigens), *F. tularensis*, RT and SEB simultaneously in human *B. anthracis* vaccinee sera (containing anti-PA and anti-LF IgG) which had been spiked with animal specific IgG antibodies to the other select agents. Inter- and intra-assay coefficients of variation were 6.5% and 13.4%, respectively (N=4). There were no significant differences ($P>0.70$) in assay responses when the assays were performed individually vs. multiplexed. When the observed vs. expected interpolated concentrations were compared using standards recovery, highly linear relationships were observed (r^2 values from 0.981 to 0.999, $P<0.001$). Minimum detectable concentrations (MDCs) ranged from 0.3 ng/ml (anti-*Y. pestis* F1 IgG) to 300 ng/ml (anti-RT IgG). Linear dynamic ranges spanned from 125 ng/ml for anti-SEB IgG to 1333 ng/ml for anti-*Y. pestis* F1 IgG. The multiplexed FCMIA is considerably faster and has extremely higher throughput when compared to existing individual enzyme-linked immunosorbent assays (ELISAs) commonly used for measurement of anti-BT IgGs. These data indicate that multiplexed FCMIA is a sensitive, accurate and precise method to simultaneously measure specific IgGs in serum to CDC select agents and may be of value in screening either decontamination workers, the general population or animals for exposures/infections to these agents.

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