CDC RESEARCH PLAN FOR INFECTIOUS VARIOLA VIRUS:
Protein-based diagnostic development
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Previous results indicate high specificity for monoclonal anti-Variola antibody E2 for antigen capture ELISA. However, greater sensitivity was observed against gamma irradiated antigen compared to a log lower response observed against live Variola antigen. Questions regarding this difference were discussed including the use of different Variola strains to generate the monoclonal antibodies and for the capture ELISA pilot. Both the mab generation and the ELSIA pilot assays used Variola strains from Bangladesh (Solamain and BSH74) that have high homology. Another question was whether or not gamma irradiation of antigen alters binding affinity on a general basis. To test this we gamma irradiated Vaccinia virus antigen to determine if different binding sensitivities were observed in a capture ELISA. In contrast to Variola, signal with live Vaccinia was higher than with gamma irradiated Vaccinia although the levels were more comparable than those seen with Variola (live versus killed). Therefore, the disparity in Variola antigen capture between live and killed antigen is not a function of different strains used or an artifact of gamma irradiation of antigen.

Extension of the antigen capture assay was performed to generate a generic orthopoxvirus antigen capture assay using polyclonal anti-sera. Using this strategy, successful detection down to 10^4 pfu of Vaccinia was observed. Application of the assay to Variola antigen has not been performed.

Additional protein based studies include the testing of a lateral flow antigen capture assay as well as development of protein microarrays for antigen characterization. Rapid lateral flow test studies were extended with use of monkeypox antigen and monkeypox clinical samples as well as vaccinia antigen. Results indicate a limit of detection of 10^5 pfu for consistent true positive readings. Variola testing has not been done to date. Protein microarrays have been developed and initiated to look specifically at Variola clonal expression and characterization of serology response to a number of Orthopoxvirus infections. To date three versions of protein chips have been used to look at orthopoxvirus serology responses against smallpox vaccine recipients, human monkeypox, convalescent Variola sera as well as sera from animal modeling of vaccinia, monkeypox and Variola infections. While the use of live Variola has not been required for these studies to date, the impact of differential reactivity using killed versus live antigen highlights the complexity of protein based detection assays. The design of this project was to allow a wider option of diagnostic methods for early detection and enhanced public health response. Outcomes from monoclonal antibody development include protein based (antigen) detection as well as the potential to derive therapeutically

valuable antibodies. Additional monoclonal antibody screening will continue against non-Variola orthopoxviruses to identify other potential candidates.