RESEARCH ABSTRACT

SWINE HEALTH

Title: Serological Approach for Diagnosis and Surveillance of Multiple Agents in Serum and Oral Fluid Samples - **NPB # 10-033**

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Scientific Abstract

Fluorescent microsphere immunoassay (FMIA), or Luminex, is a relatively new technology for serologic diagnosis of infectious disease. Advantages over standard technologies, such as ELISA, include the detection of multiple targets in a small (50 ul) sample with greater sensitivity and specificity, and at a relatively low cost. This technology can be adapted for use on non-serum samples such as oral fluid. Previous Luminex technology, e.g. Bio-plex, incorporated fluorescently labeled polystyrene beads coated with antigen combined with flow cytometry to detect bead types and bound antibody. Just recently, a new instrument, MAGPIX, was introduced by Luminex Corp. The technology incorporates magnetic microspheres and LED detection. The new instrument is lower in cost, possesses a smaller footprint and has simplified sample processing protocols. The magnetic beads can be used in the Bio-Plex instrument. The objectives of this project were to (1) to develop a multiplex fluorescent microsphere immunoassay (FMIA) or "Luminex" for the detection of PCV2, PRRSV, SIV, and *M. hyo*-specific antibodies in serum and oral fluid samples and (2) to validate the FMIA for incorporation into a single multiplex diagnostic platform. A third objective, added during the project, was to adapt the FMIA to the new MAGPIX format. This modification created the opportunity to incorporate a format likely to be used by veterinary diagnostic labs. Viral proteins were expressed as 5xHis-ubiquitin fusion proteins in *E. coli* and affinity purified on a nickel column under native conditions. Purity was verified by SDS-PAGE and total concentration measured by Biorad assay. *M. hyo*. proteins were prepared as a Tween-20 lysate. Except for the Tween-20 lysate, all proteins were successfully conjugated to the MAGPIX beads. The antigens included N protein from type 1 and type 2 PRRSV, NP and NS1 from swine influenza virus, CP(43-233) and CP(160-233) from PCV2. All antigen targets were assembled into a single multiplex and tested against sera known to be positive for antibody, i.e., infected with PRRSV, PCV2 and SIV. All antigens possessed the predicted antibody specificity using sera with known reactivity. Initially, assays were performed with the Bio-Plex and later with the MAGPIX. Results for both instruments were similar. Samples from 200 pigs experimentally infected with PRRSV were tested with both Luminex and IDEXX. The results for days 4, 7 and 11 showed agreement with the two assays, except that the MAGPIX identified more samples as positive, indicating a greater sensitivity for the Luminex assay. Results for the IgM assay showed the presence of PRRSV-specific antibody for the first 21 days after infection.