SWINE HEALTH INFORMATION CENTER
INTERIM RESEARCH GRANT REPORT FORMAT

I. Project Title and project identification number:

Development of direct detection methods for *in situ* diagnostic of Seneca A virus

Principal Investigator:

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Institution:

Iowa State University

II. Stated Objectives from original proposal

The specific aims of this proposal are to develop a set of direct diagnostic tools that allows direct detection of SV-A *in situ*.

A. Development of SVA immunofluorescence assay (IFA) for the detection viral antigen in infected cell culture (*completed*).

B. Development of SVA-IHC for detection of viral antigen in clinical specimens (*currently under development*).

C. Development of SVA *in situ* hybridization (fluorescent and/or chromogenic) for direct visualization of viral nucleic acid in clinical specimens *(completed)***.**

PRODUCER UPDATE:

A. Development of SVA immunofluorescence assay (IFA) for the detection viral antigen in infected cell culture **(*Completed*).**

This objective has been completed on time. The main idea was to develop a tool that allows us to confirm the virus in cell cultures. The development of this technique has a tremendous impact for confirmation of virus isolation. Once SVA is isolated from clinical samples direct IFA is the technique of choice to confirm the presence of the virus. Basically, we will be able to stain infected cell having a more objective way to confirm infection through viral staining with specific antibodies (figure 1). As compared to a PCR assay, this allows us to identify presence of LIVE virus.

For this specific objective we developed reagents that were not commercially available and now are not only available for ISU Veterinary Diagnostic Laboratory's diagnostic use, but for researchers and other diagnostic laboratories as well.

B. Development of SVA-IHC for detection of viral antigen in clinical specimens **(*Currently under development*)**.

This objective has been partially achieved. We proposed to develop a technique that allows us to detect SVA in fixed tissues. Since SVA vesicular lesions are non-specific, this technique will allow us detect the virus in lesions and allows us to differentiate SVA from other potential causes of vesicular disease. In order to achieve this goal, we proposed to generate two different types of antibodies (polyclonal and monoclonal). The development of a

polyclonal is complete and already evaluated with excellent results (Figure 2). We are able to detect SVA in section of skin with vesicle. Polyclonal antibodies are easier and faster to produce, but they have less specificity than monoclonal antibodies; thus, they may allow for some cross-reaction and can be more difficult to interpret.

In order to provide a more refined diagnostic tool we also proposed to develop a monoclonal antibody. This technique is still under development. The development of this reagent is done in mice and takes approximately 3 months to complete the process, including multiple steps that cannot be accelerated: the mouse must produce sufficient antibodies to test, and as this is an immune response, takes time to build. Unfortunately, our first attempt provided poor quality antibodies therefore we are repeating the production of new candidate monoclonal antibodies to achieve maximal applicability. We are in the process of screening and evaluating the new SVA-Mab.

For this specific objective we developed reagents that were not commercially available and now are not only available for ISU diagnostic laboratory but for researchers and other diagnostic laboratories.

C. Development of SVA *in situ* hybridization (fluorescent and/or chromogenic) for direct visualization of viral nucleic acid in clinical specimens **(Completed).**

This objective has been completed on time. We evaluated a set genetic probes that allows us to confirm the presence of the virus in tissues based on the presence of genetic material. We evaluated two set of fluorescent probes targeting different regions of the virus. One of them (VP1) showed to be adequate to detect SVA genetic material in tissues. (Figure 2, 3). In order to reduce the effort and cost involved in fluorescent detection, we further evaluated this probe for detection of SVA detection material with light microscopy. SVA VP1 probe was shown to be efficient detecting viral genetic material with light microcopy. This technique will allow us to detect SVA efficiently in lesions without the burden of expensive fluorescent scopes. The benefit of this over PCR is that it will allow us to detect the exact location of virus, which will help to understand where and how long the virus persists in tissue.