SHIC – FINAL RESEARCH GRANT REPORT

Project Title and project identification number: Development of direct detection methods for in situ diagnostic of Seneca A virus (SHIC Project # 15-195)

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Industry Summary: The specific aims of this proposal are to develop a set of diagnostic tools that allows direct detection of SVA.

The first objective was to develop and evaluate a SVA immunofluorescence assay (IFA) for the detection virus in cell culture. 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. Thus, we were able to stain infected cells having a more objective method to confirm infection. In addition, this method, compared to a PCR assay, allows us to identify presence of viable 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.

Our second objective was to develop a technique that allows identification of the virus in clinical specimens fixed in formalin. Since SVA vesicular lesions are non-specific, this technique is important to detect the virus in lesions and differentiate SVA from other potential causes of vesicular disease. We generated two different antibody reagents that can be used to detect SVA in sections of skin with vesicular lesions. These two antibodies were not commercially available and are now not only available for ISU diagnostic laboratory but for researchers and other diagnostic laboratories.

The third objective was to develop a technique that allows visualization of viral genetic material in clinical specimens. This technique uses fluorescent molecular probes that target two different regions of the virus. In order to reduce the effort and cost involved in fluorescent

detection, we further evaluated this probe for detection of SVA with light microscopy. This technique will allow efficient detection of SVA in lesions without the burden of expensive fluorescent scopes. The benefit of molecular detection of SVA in tissues over viral detection by PCR is that can we can also demonstrate viral location in tissues, which will help to understand where and how long the virus can persist in tissues.

In conclusion, we successfully developed a set of reagents that can be used in different diagnostic techniques for virus identification in tissue. These techniques will have a great impact on SVA diagnosis in cases of vesicular disease, providing and supporting the differential diagnosis with other causes of vesicular disease such as foot and mouth disease.

Keywords: Senecavirus A, Immunohistochemistry, Monoclonal antibodies, *In situ* hybridization, Vesicular disease