A New Diagnostic Test for the Detection of Bovine Leukemia Virus (BLV) Infection in Cattle

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Introduction

Bovine leukemia virus (BLV) is a retrovirus infection of both dairy and beef cattle known to cause bovine leukosis and malignant lymphoma. Clinical signs of the disease, such as weight loss and decreased milk production, yield economic losses. Moreover, trade restrictions are placed on positive animals. Since there is no vaccine or treatment, biosecurity measures can help control the spread of the disease. Current testing methods, although highly sensitive and specific, require the practitioner to send in samples to a specialized laboratory. The turnaround time for results can be on the order of a few days to a few weeks. Tip-Test™: BLV (ImmuCell Corporation, Portland, Maine) has developed an on-site, rapid serological test for the presence of anti-BLV antibodies. The test is based on the enzyme-linked immunosorbent assay (ELISA) principle, yet provides results in 25 minutes and can be used with whole blood, serum or plasma. This self-contained kit requires no specialized equipment or laboratory and can be used by practitioners in the clinic or on-site.

Materials and Methods

The Tip-Test™: BLV is a self-contained test with four reagent wells, and a tip in a plastic tray. The tip is a pipette tip containing three porous filter elements that allow fluid flow across the large surface area inside each element. The top element is a positive procedural control; the center element is the negative control, providing baseline color to compare to the test element; and the bottom element is the test element with bound BLV antigen. A positive result causes a purple color to develope on the test element, while the negative element remains a white-to-slight-gray color.

The Tip-Test™: BLV procedure is as follows: (1) A sample is diluted in sample diluent (well #1) and is incubated for five minutes. (2) The diluted sample is then drawn into the tip and mixed. (3) After five minutes, the sample is expelled and conjugate (well #2) is drawn into the tip and incubated for five minutes. (4) The tip is then washed (well #3). (5) Substrate (well #4) is drawn into the tip and incubated for five minutes. (6) The wash step is then repeated and the result is read with the aid of a "comparator card". To facilitate sample throughput during the clinical trial, a multichannel format of the test was implemented.

Results and Conclusions

At three independent sites, 100 serum samples—composed of 50 BLV positive and 50 BLV-negative, and from a herd managed for low seroprevalence of BLV—were coded and run in a blinded fashion. One of the sites also ran the commercially available Agar Gel Immuno-Diffusion (AGID) as the gold standard for comparison, as well as a commercial ELISA test. Tip-Test™: BlV demonstrated an average sensitivity of 100% and an average specificity of 98.7% by comparison with the AGID test. There was 99% agreement with the ELISA results. The analytical sensitivity was compared between Tip-Test™: BLV and the ELISA assay, whereby a single BLV-positive serum was titrated and assayed. Both assays generated cutoffs between 1:4000 and 1:8000.

The high sensitivity and specificity, coupled with the ability to perform this test at the clinic or on-site with results in only 25 minutes, make Tip-Test™: BLV an important tool for diagnosing BLV infection in cattle. This allows producers and veterinarians to implement effective herd management decisions quickly and improve chances of controlling the spread of BLV.