# Evaluation of the Svanovir™ Enzyme-linked Immunosorbent Assay (ELISA) for Bovine Leukosis, Bovine Viral Diarrhea and *Mycobacterium paratuberculosis*

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## Introduction

Three novel serologic tests (SVANOVA Biotech, Uppsala, Sweden) were evaluated: an indirect ELISA for Bovine Leukosis Virus (BLV), an indirect ELISA for Bovine Viral Diarrhea (BVD), and an indirect ELISA for *M. paratuberculosis* (Johne's Diease).

## Materials and Methods

Each test was compared to industry standards in North America: IDEXX ELISA for BLV and Johne's (IDEXX, Westbrook, Maine USA) and serum neutralization (SN) for BVD.[2] Where appropriate, sensitivity and specificity values or Kappa scores were calculated. Serum samples were collected as part of a seroprevalence survey for production limiting diseases in Eastern Canada. Thirty lactating cows in each of 30 herds in Nova Scotia, New Brunswick and Prince Edward Island were sampled. Both herd and cow selection were by formal random sampling. From this serum bank, 528 cows were selected for the comparative BLV testing, 498 cows for *M. paratuberculosis*, and 218 unvaccinated animals, greater than 6 months of age, for BVD testing.

## Results and Discussion

Table 1 is a contingency table of the BLV results. Serologic diagnosis of infection with BLV is very accurate.[3] Therefore, sensitivity and specificity could be calculated. Since there were no false positives or false negatives, the test scored 100% on both parameters.

Table 2 contains the BVD results. SN is considered the gold standard for BVD. When compared to a SN dilution ≥1:256, the sensitivity of the ELISA was 94% and the specificity 96%.

**Table 1.** SVANOVA and IDEXX BLV

| | IDEXX +ve | IDEXX -ve | Total |
|---|---|---|---|
| Svanova +ve | 225 | 0 | 225 |
| Svanova -ve | 0 | 303 | 303 |
| Total | 225 | 303 | 528 |

**Table 2.** SVANOVA and SN BVD

| | SN ≥ 1:256 | SN ≤ 1:256 | Total |
|---|---|---|---|
| SVANOVA +ve | 33 | 7 | 40 |
| SVANOVA -ve | 2 | 176 | 178 |
| Total | 35 | 183 | 218 |

**Table 3.** SVANOVA and IDEXX Johne's

| | IDEXX +ve | IDEXX -ve | Total |
|---|---|---|---|
| SVANOVA +ve | 22 | 83 | 105 |
| SVANOVA -ve | 5 | 388 | 393 |
| Total | 27 | 471 | 498 |

Table 3 contains the Johne's results. Reported IDEXX sensitivity is 47.3% and specificity is 99%.[1] There was a relatively low agreement between tests (Kappa 0.27) beyond what is expected due to chance alone. It is interesting to note, however, that the SVANOVA test identified many more animals as positive, which may be an improvement on the high false-negative rate of the IDEXX test.

### Conclusions

The SVANOVA ELISA for BLV and BVD appear to be equivalent to industry standards. The SVANOVA ELISA for *M. paratuberculosis* shows some promise of improvement on current North American testing methods. Additional evaluation of this test is necessary to discern its full capabilities.

### References


1. Collins MT, Sockett DC, Ridge S *et al*: Evaluation of a commercial enzyme-linked immunosorbent assay for Johne's disease. J Clin Microbiol 29:271-276, 1991.
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3. Miller JM: Comparison of four serologic tests for the detection of antibodies to Bovine Leukemia Virus. Am J Vet Res 42:5-8, 1981.


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# Comparison of Two Assay Techniques to Study *In Vitro* Phagocytosis of Bovine Granulocytes Using Flow Cytometry


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### Introduction

The response of phagocytic cells to *Pasteurella haemolytica* is difficult to study *in situ*. Available assays to study the function of these cell types often do not accurately reflect the physiology of the cell in response to bovine pasteurellosis. This study compared granulocyte phagocytosis of fluorescent beads and fluorescent bacteria using flow cytometry. Development of an *in vitro* assay would provide a model to study nutrient and pharmaceutical effects on the immune response to *Pasteurella* infections. It would also allow for future studies of the pathogenesis of not only bovine pasteurellosis but other diseases.

### Materials & Methods

*Pasteurella haemolytica* was cultured, harvested and conjugated with 5,6-carboxyfluroescein, succinimidyl ester (5,6-FAM-SE). Labeled bacteria was quantitated using a flow cytometric technique and snap frozen at –70°C until time of assay. Whole blood was collected via jugular venipuncture into 10 ml sodium heparin Vacutainer™ tubes on three consecutive days from 10 bull calves, 10 heifer calves, and 10 bull calves respectively. Blood obtained from all 30 calves was used to test two *in vitro* phagocytic assay techniques.

Isolated leukocyte cell populations were prepared and stimulated, in triplicate, with fluorescent-labeled synthetic beads and labeled *P. haemolytica* to measure phagocytic cell activity. Uptake of the fluorescent polystyrene beads and 5,6-FAM-SE-conjugated bacteria by phagocytic cells was evaluated by flourescent emission using a Coutler EPICS XL flow cytometer. Variance components were estimated separately for each assay. The MIXED procedure of the SAS system was used to fit a random effects model and the GLM procedure was used to perform an analysis of variance comparing percent labeled cells for each assay.

### Results and Conclusions

The bacteria assay labeled a higher percentage of cells, at 44.9%, than the bead assay at 19.4% ($P<0.001$). Estimates of variance components, including day, calf and sampling, are shown in Table 1. The higher value measured for uptake of bacteria, and greater variability of the bacteria assay, caused a large and non-constant lack of agreement between the two assays.