Testing of cattle ear notch samples using a Bovine Virus Diarrhea antigen ELISA kit

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1. Introduction
With ELISA, the BVDV detection of antigen can be done by looking for all virus antigens, for p80 only (non structural protein highly conserved among strains) or for the glycoprotein E₀. This last one is also named gp48 or E₉s in the updated nomenclature. It can be found in skin epithelial cells as in other tissues from infected animals (Haines et al., 1992; Njaa et al., 2000). This property is used by ImmunoHistoChemistry (IHC) performed on skin biopsy as ear notches. Ear notch samples can be obtained easily and quickly, and require less skill to obtained than blood sample.

Another important advantage of the ear notch skin biopsy samples is that they contain a high concentration of BVDV antigen, but only a relatively small amount of blood. The presence of maternal anti-BVDV antibodies therefore does not interfere with the detection of the BVDV antigen in the ear notch sample as it does with serum or plasma samples. So calves can be tested before the age of 3 months.

2. Material et methods
The samples used in this study were from veterinary diagnostic labs at Cornell university, University of Nebraska, and Oklahoma State University. Reference method BVDV testing (virus isolation, PCR, or IHC) was performed by each of these labs on their own samples. 100 samples from calves ranged in age from 1 day to adult (more than 3 months) were tested by reference technique in the following proportions: 61 by IHC, 23 by PCR, 10 by virus isolation on buffy coat, 5 by virus isolation on sera and 1 by ELISA on serum.

The kit tested in this assay (SERELISA™ BVD E₀ Ag Mono Indirect, Synbiotics Europe) was evaluated on ear notch samples. This is an indirect mono well antigen ELISA. When performed on serum the ELISA was carried out according to directions in the kit enclosure. Ear notch approximately 1cm x 1cm in size was cut from the edge of the ear of each subject animal. Each of the samples were mixed to 2mL of phosphate buffered saline pH 7.4 then allowed to soak in PBS for a minimum of 10 minutes at room temperature. The ELISA was carried out exactly as described in the kit enclosure, except that 100 µL of the ear notch/PBS supernatant was pipetted into each reaction well in place of serum. The data reduction and the cut-off’s for the ear notch samples were the same as detailed in the kit enclosure for serum samples.

Complementary studies on the ELISA kit using ear notch samples were performed: relationship between size of ear notch and assay results for three representative BVDV positive samples, as well as effect of soak time of this three ear notch samples in PBS.

3. Results
The cattle included in this study ranged in age from 1 day to adult (more than 3 months). This included 60 young animals (less than 3 months), 47 of which were from 1 day old pre colostral calves. All results obtained with ELISA on ear notch samples correlate perfectly with results obtained with reference technique.
Observed correlation is 1 (100/100 animals). Sensitivity index of the ear notch/ELISA was 100% (39/39) relative to the reference methods, and specificity index of the ear notch/ELISA was 100% (61/61) relative to the reference methods. At a risk $\alpha=5\%$, the minimum guarantied indexes are 91% for sensitivity and 94.1% for specificity.

Data obtained from relationship study between ear notch size and assay result are positive. No significant decrease was observed on BVDV-positive ear notches down to 0.25cm x 0.25cm in size.

Data obtained from relationship study between soak time in PBS (from no soak time to 10 days of soaking) and assay results are all positive. Even with no soak time the results obtained are clearly positive.

4. Discussion
The excellent agreement between ear notch/Ag ELISA results and the reference methods on these samples indicates that no age limitations are necessary when assaying ear notch samples by antigen capture ELISA. This is not the case when this ELISA is performed on serum samples: this is due to the potential interference of anti-BVDV maternal antibodies (Palfi et al., 1993). These circulating maternal antibodies would not be expected to be present at a appreciable levels in the case of these ear notch samples. This is supported by the results obtained with sample from a 1.5 week old persistently infected animal. This animal was BVDV positive by PCR and by ELISA performed on ear notch but negative by ELISA performed on serum.

The recommended size of 1cm x 1cm for ear notch represents an adequate margin of safety to insure that BVDV-positive samples are correctly identified. This size is four times more than one which keeps gives significant positive results.

The results obtained with different soak times give significant positive results even with no soak time protocol. But when soaking time increase, the value obtained with ELISA do the same. Therefore the recommended 10 minutes incubation period represents a compromise; it is short enough to be convenient, yet long enough to insure adequate solubilization of antigen

5. Conclusion
The testing of ear notch samples with the antigen capture ELISA tested here and utilizing the procedure described in this study represents a very effective means of assessing the BVDV-status of cattle of all ages, especially young ones under colostral protection. This test is an attractive second choice to expensive and difficult techniques that were the only available for testing young animals less than 3 months of age with suspected presence of maternal antibodies.

References