Obtaining Quantitative Data Using VMRD's cELISAs

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It is often useful to know the titer of antibody in a serum for comparison with sera taken earlier or later from the same animal or for other purposes. The cELISAs made by VMRD recommend using undiluted sera and the results are scored as positive or negative. These assays are adjusted to detect low as well as high amounts of antibody. Therefore, moderate amounts of specific antibody will cause maximal % inhibition preventing using the % inhibition to differentiate sera with moderate and high antibody. Of course, sera with low amounts of antibody that cause % inhibitions near the cutoff have less antibody than sera with higher % inhibitions. However, sera with similar % inhibitions near maximal for a particular cELISA can have very different amounts of antibody. These cELISAs can easily be turned into very quantitative tests by evaluating serial dilutions of a serum. The endpoint can be determined for each dilution by scoring each dilution tested as positive or negative based on the % inhibition cutoff recommended for the kit being used.

For instance, using the CAEV cELISA to test dilutions of 1:10, 1:100, 1:1000 and 1:10,000 of an infected goat's serum results in a >60% inhibition at 1:10, 1:100 and 1:1000 dilutions with the 1:10,000 dilution causing <35% inhibition. Since the recommended cutoff for a negative test in the CAEV cELISA is 35% or less, then the titer of the serum is 1:1000 in this example. A similar procedure can be used to obtain antibody titers using the *Anaplasma*, *Neospora, Babesia equi, Babesia caballi* and Bluetongue virus cELISAs.

One issue to keep in mind when determining antibody titers using these cELISAs is that antibody is being measured to a single epitope present on a single protein. This is because a positive assay for antibody in all these tests depends on the antibody in the serum of interest inhibiting the binding of a monoclonal antibody. The monoclonal antibodies are directed to an epitope on a protein or glycoprotein of the organisms for which antibody is being used. Detecting antibody to a single epitope is not a problem since the monoclonal antibodies in each test were selected because sera from infected animals inhibited their binding. This is the basis of the usefulness of these cELISA's to detect specific antibody in infected animals.