

## Reasons to Avoid the CF Test for Detecting Antibodies in Cattle, Sheep, Goat and Horse Sera

Travis C. McGuire, D.V.M., Ph.D., Director of Research | Apr 2005

The complement fixation (CF) test is sometimes used for detection of antibody to infectious agents in domestic animals. There is a major problem with the use of this test to detect antibodies in serum for cattle, sheep, goats and horses. The problem is false negative reactions. These are caused by failure of antibody of certain isotypes to fix guinea pig complement which is the complement source used in standard CF tests. The presence of antibody of a non-complement-fixing isotype will result in a negative CF reaction even though antibody is present.<sup>1</sup> The problem is further compounded because non-complement-fixing antibody competes with the complement-fixing antibody for antigen. This also results in a negative reaction when, in fact, antibody is present.<sup>2</sup>

It is bovine, sheep and goat IgG2 isotype antibody that does not bind guinea pig complement and this isotype is present in large quantities in the sera of these animals. That bovine IgG2 antibody fails to bind complement was first described in 1966 and verified in at least four other manuscripts (reviewed in reference 1). The same observations have been made with sheep and goat IgG2 antibodies. In horses, IgG(T), which is a major immunoglobulin isotype in serum, does not bind guinea pig complement (reviewed in reference 2).

The severity of the problem of using CF for detection of cattle serum antibodies is clearly illustrated in a publication on detection of antibodies to *Anaplasma marginale*. Sera from 232 cattle were defined as *A. marginale* positive or negative by nested polymerase chain reaction methods and hybridization and evaluated for antibody using the CF test.<sup>3</sup> The best estimate of the CF test sensitivity was 20%; this means that 80% of the *A. marginale* positive cattle were not detected. A cELISA detected 98% of the *A. marginale* positive cattle from the same cohort of cattle.<sup>4</sup> The cELISA detects antibodies of all isotypes. It was concluded from these studies that the CF test was ineffective for identifying cattle persistently infected with *A. marginale*.<sup>3</sup>

A problem of similar magnitude has been documented in horses. Horses infected with equine infectious anemia virus (EIAV) are infected for life and have detectable antibody for life. However, the CF test is positive early after infection, but then becomes negative.<sup>2</sup> Purified IgG isotype antibody from the CF negative sera of infected horses was positive in the CF test. Purified IgG(T) isotype antibody from the same CF negative sera from infected horses was negative in the CF test. Mixing the purified IgG(T) and IgG isotype antibodies resulted in a negative CF test.<sup>2</sup> These observations provide an explanation for why the CF test with whole serum obtained later in EIAV infection is negative even though antibody to EIAV antigen is present and detectable by agar gel immunodiffusion which detects antibody of both isotypes. Based on these documented problems, the CF test should be avoided when the aim is to detect antibodies to infectious agents in cattle, sheep, goats and horses. Further, if the CF test is used, there will be very little correlation with the sensitivity obtained using other tests including ELISA, cELISA, immunofluorescence, and western blot that are not negatively affected by certain isotypes of the antibody.

1. McGuire, T.C., *et al.* Functional properties of bovine IgG1 and IgG2: Interaction with complement, macrophages, neutrophils and skin. *Immunology* 38:249-256, 1979.

2. McGuire, T.C., *et al.* The complement-fixation reaction in equine infectious anemia: Demonstration of inhibition by IgG (T). *J. Immunol.* 107:1738-1744, 1971.

3. Bradway, D.S., *et al.* Sensitivity and specificity of the complement fixation test for detection of cattle persistently infected with *Anaplasma marginale*. *J. Vet. Diagn. Invest.* 13:17-81, 2001.

4. Torioni de Echaide, S., *et al.* Detection of cattle naturally infected with *Anaplasma marginale* in a region of endemicity by nested PCR and a competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5. *J. Clin. Microbiol.* 36:777-782, 1998.