Lyme Quant-c6 assay vs AccuPlex4 Lyme assays
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The development of the new AccuPlex4 assay by Antech Diagnostics has produced multiple questions regarding the diagnosis, interpretation and clinical management of clinical, sub-clinical and non-clinical lyme borreliosis in dogs. The new assay has also exposed several topics of confusion, misinterpretation and false assumptions regarding previously employed diagnostic assays for both patient side testing and reference lab confirmation.

More specifically, the AccupPlex4 assay is often compared and contrasted to the Snap 4DX and Idexx Quant c6 assays. All three assays measure the antibody response to Borrelia burgdorferei exposure and previous infection. The AccuPlex4 assay utilizes protein markers to identify previous vaccination with all OspA containing canine vaccines along with 4 additional proteins to identify and differentiate acute vs chronic exposure/infection to the agent. The Snap 4Dx and Quant-c6 are similar to each other and measure antibodies against the chronic marker synthetic c6 peptide derived from the IR6 region of the Vsle protein to the agent. Both the Snap 4DX and the Quant-c6 measure the same antibody with the quant-c6 test also providing an optical density reading from the microwell ELISA assay.

Several false assumptions and misconceptions exist around the marketing and clinical application of the Quant-c6 assay. The Quant-C6 assay is not a serological titer but merely an optical density reading from an elisa plate reader reflective of how much "blue" color is in the well. This is analogous to saying that a Snap 4Dx kit blue dot is "faintly blue", "somewhat blue", "blue" or "dark blue". The false assumption is that the degree of "blue" or C6 antibody optical density number is always proportional to antibody level in serum and has clinical significance related to actual Lyme disease and or clinical response to therapy and clinical outcome. This concept has never been proven or validated in veterinary and/or human medicine. The Quant-C6 test was developed as an add-on reference lab tool to help resolve the problems and complaints associated with questionable, vague and difficult to interpret in-clinic Snap 4Dx Lyme results. Unfortunately and despite the misperceived application, initial or monitored levels of anti-lyme antibody (c6 or any other marker) have not been shown to correlate with disease severity, clinical signs, therapeutic success or eventual clinical outcome. The ACVIM consensus statement (which includes input from Drs. Lappin, Goldstein, Littman) states there is no clinical benefit for using any measurement of quantitative antibody levels (including C6) for the treatment, diagnosis or clinical evaluation of Lyme disease in veterinary medicine.

The only published article on Quant-c6 assay in dogs was by Dr. Levy and the Idexx marketing department in 2008. The article took approximately 68 dogs with positive snap results and subsequent quant-C6 numbers. The Quant-c6 results had no correlation with clinical signs, presentation, tick exposure, disease etc. The authors treated approximately 53 patients leaving 15 as controls and arbitrarily set a cutoff for high vs low C6 values (once again the cutoff level was not related to clinical disease). They dogs with higher C6 values tended to show a larger percentage decrease in the follow-up optical density values at 6 or 12 months vs the untreated dogs; however, the untreated group also showed spuriously lower numbers. The dogs with

lower C6 values showed a lower percentage decrease similar to the untreated. There was little change in C_6 level following antibiotic therapy in the 23 dogs with low initial C_6 levels.[1] The only viable conclusion available from this study is that dogs with higher C6 optical density values occasionally show a greater or faster decrease in optical density values compared to dogs with initially lower values or untreated dogs at one year post treatment. The study has no correlation with clinical signs, improvement, disease recrudescence, clinical success or potential future immune mediated disease including lyme nephropathy.

A human study looking at actual C6 serial dilution titers in 2005 showed that monitoring antibody titers as a potential guide to therapeutic success is only helpful with acute and peracute localized disease when associated with erythema migrans in the first 35-40 days post exposure.[2] The chronic exposure patients (which is the usual presentation in dogs) showed a more variable response in antibody titers following antibiotic therapy along with variable clinical improvement. The Snap 4Dx and Quant-6 assays only measure the antibody response to a single subacute to chronic phase marker of the disease process.

Subsequently, from these papers and the human lyme literature, clinical differentiation between acute vs chronic exposure (as reported with the AccuPlex4) is possibly and potentially a more accurate tool for immediate aggressive therapy vs a c6 arbitrary number or optical density reading. The c6 antibody assay is a good assay for detecting chronic exposure to Borrelia burgdorferci; however, no evidence exists to suggest the level of color change for the assay has any remote correlation to successful therapy, clinical improvement or future clinical progression. Lyme disease is extremely complex and we as veterinary clinicians are naive to believe we can monitor or use an arbitrary plate reader number as an indicator of therapeutic success or clinical improvement. Clinicians need to combine the AccuPlex4 results with clinical signs, other laboratory data (microalbuminuria, Urine protein:creatinine levels, hematology data, chemistry results, synovial fluid cytology etc), history and evidence of tick exposure to help determine the clinical impact of acute vs chronic disease. Any patient with clinical evidence of disease should be treated. Doxycycline therapy may also be considered with acute exposure results according to human medicine. Therapy for chronic cases should be directed toward the clinical presentation to determine if doxycycline, corticosteroids or both are needed. In general, acute exposure should progress to a chronic or negative result at 12 months post treatment unless re-exposed or re-infected with the agent. In patients with chronic exposure/infection antibodies to Borrelia burgdorferei, a follow-up urine microalbumin or urine protein:creatinine level is a much more valuable tool than a Quant-c6 arbitrary "blue number" value.

The Bio-CD technology utilized by the AccuPlex4 assay, has the inherent technical capability to provide a number proportional to the amount of antibody present in serum for any of the 5 markers to Borrelia burgdorferci utilized in the assay. In fact, no additional tests would need to be run to provide this value. Antech has chosen not to currently provide a numerical option since the current scientific literature and data from both veterinary and human medicine indicates the numbers may have no clinical value with regards to the treatment or monitoring of clinical borreliosis. If future research in borreliosis shows the antibody levels can be used to correlate with clinical therapeutic success, therapeutic monitoring or severity of disease, then at that time numerical reporting would be indicated to go along with the positive or negative reports for acute and chronic exposure to Borrelia burgdorferei. One concern is that this type of data could

currently be clinical misleading (as potentially with the Quant-C6) versus truly valuable clinical data and information. No data is better than misleading data!

References:

1) Clinical and Vaccine Immunology, January 2008, p. 115-119, Vol. 15, No. 1
2) Clinical and Diagnostic Laboratory Immunology, Sept. 2005, p. 1069–1074 Vol 12, No 9