

# L.16.- Counter Immuno Electrophoresis: Methodology for confirmation of positive results on Immunoassay and DNA based tests through detection of milk based proteins

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Following the outbreak of Bovine Spongiform Encephalopathy (BSE) the inclusion of animal proteins in compound animal feedstuffs was restricted. Banned proteins are generally associated with rendered material in which most proteins are significantly disrupted and denatured. Some animal proteins are permitted e.g. milk products. These are not subjected to the same heat exposure as rendered material and retain aspects of their neo-protein structure. Problems arise when permitted feed ingredients generate positive reactions on immuno assay and DNA based tests. i.e. a positive bovine result could be due to the presence of meat and bonemeal contamination but could also result from the presence of permitted milk products. Testing milk products using a counter-immuno electrophoresis technique, with antisera raised against non-heat treated serum based proteins, confirmed that non-denatured proteins were present. This was not the case when testing rendered material where the protein structure is altered far beyond the detection capability of a serum based test. Non-denatured proteins, such as those derived from milk products, can be extracted from within a compound feed matrix using an ammonium sulphate precipitation process (precipitation band 40% to 77% w/v). Problems associated with the liberation of proteins from within feed pellets can be overcome by soaking samples in a buffer heated to 50°C (this temperature and time exposure isn't sufficient to inactivate the test). Extracted feed pellets are tested using counter-immuno electrophoresis. This test is based on electro-osmosis in an alkaline gel (pH 8.6) and tested using species specific antisera. This test can be used alongside assays for the detection of rendered materials (ELISA or PCR) in order to rule out milk powders as the cause of positive reactions, e.g. a positive ELISA test coupled with a negative CIE test would strongly suggest that a positive result would not be a consequence of permitted material within the feedstuff. Data is presented outlining test development, sensitivity and application of this method.

## **Keywords :**

*Counter Immuno Electrophoresis*