

A NON-CONVENTIONAL SOLID PHASE FOR IMMUNORADIOMETRIC  
ASSAYS OF POTENTIAL USE IN THE DIAGNOSIS OF  
CHAGAS' DISEASE.

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Since the silanol groups of silica particles can be chemically modified by reacting them with 18 carbon lipidic chains, the adsorption surface of the particle not only increases significantly, but also becomes highly hydrophobic. This modified material, octadecyl silica or Si-C18, displays a strong binding capacity for peptides, proteins and other molecules with hydrophobic domains. For these reasons, it occupies a dominant position as stationary phase in high pressure liquid chromatography (HPLC). In order to evaluate of the Si-C18 solid phase in a two antibody-two site TRMA, we attempted the detection of a plasma protein.

The chosen molecule was the murine sex-limited protein or Slp, a 200 kD, non-functional product of a gene duplicated from that coding for the fourth component (C4) of the mouse complement system. Both genes are tightly linked within the H-2 complex. (Ferreira et al 1978).

The Slp is specially suitable for this comparison, since two monoclonal antibodies, directed against different epitopes of the molecule are available (Ferreira et al, 1982). In addition the levels of Slp in plasma are genetically controlled thus providing ideal negative and positive controls for the comparative IRMAs.

Depending on the allele present in the Slp locus, the animals will (Slp<sup>a</sup>) or will not (Slp<sup>0</sup>) express the protein in their sera. The name (sex-limited protein) derives from the fact that, in general females from an Slp<sup>a</sup> strain express only 0.1 -5% of the levels present in males. (Ferreira et al, 1982).

When the signals obtained with A/J (Slp<sup>a</sup>) males were compared with those obtained with the B10 (Slp<sup>0</sup>) negative control, it was concluded that 1 mg of Si-C18 is at least as sensitive as a PVC well.

One mg of Si-C18 can adsorb between 5 and 14 times more protein than the PVC and the saturation capacity of 1 mg of Si-C18 is about 30 times larger than that of one PVC well. Obviously, this capacity could be increased at will if variables such as amount of Si-C18, volume and concentration of the protein solution are properly calibrated. Moreover, 1 mg of Si-C18 contains approximately  $4 \times 10^6$  beads, with a total surface of around  $50 \text{ cm}^2$  (the surface of a PVC microtitration well is about  $1 \text{ cm}^2$ ). The Si-C18 also compares favorably, in terms of surface, with the polystyrene beads of 0.64 cm in diameter (Ziola *et al*, 1977), used for the detection of molecules such as interferon gamma, (Chang *et al*, 1984).

In systems where low concentrations of antigens are present in large volume of complex liquid biological specimens (*i.e.*: serum, urine, ascites, spinal and pleural fluids, sputa, etc.) the Si-C18 may present advantages over the PVC plates and it may have potential value in the diagnosis of some parasitic diseases such as Chagas'. It is conceivable that the Si-C18 matrix saturated with monoclonal antibodies may represent an exceptional probe to rescue antigenic moieties from such fluids. The final detection of these moieties may be accomplished with a second

radiolabeled or enzyme-coupled antibody. Another possible use of the Si-C18 could be the detection of specific antibodies present at low concentrations in large volumes of biological fluids. For this purpose, the beads should be covered with the antigen and used to capture specific antibodies which could be detected with a radiolabeled or enzyme-coupled anti-immunoglobulin.

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REFERENCES

Ferreira, A., et al. (1978). *J. Exp. Med.* 148: 1186.

Ferreira, A., et al. (1982). *J. Immunol.* 129: 1506.

Chang, T. W., et al. (1984). *Proc. Natl. Acad. Sci.*  
U.S.A. 81: 5219.