

Abstract

An improved enzyme-linked immunosorbent assay (ELISA) for detection of heterophile Hanganutziu-Deicher (HD) antibodies and antigens, which are frequently detected in sera and/or cancerous tissues from patients with various cancers was developed using biotinylated chicken anti-GM3(NeuGc) antibody and avidin-horseradish peroxidase conjugate. The N-glycolylneuraminyl-lactosyl-ceramide, GM3(NeuGc) ganglioside was purified from horse erythrocyte membranes. The ELISA procedure required 300 ng GM3(NeuGc) antigen to coat plastic microtiter plates and 190 ng biotinylated antibody per well to give optimum product formation. The technique could detect 6 ng antigen in tissue homogenate as compared to 0.6 ng of the pure compound by inhibition. Chicken anti-GM3(NeuGc) antibody quantitatively inhibited the biotinylated antibody, however, this procedure was not suitable to quantify lower affinity HD antibody in patient sera. Immunostaining specific for HD antigen-positive cells, in tissue sections was by 4 micrograms/ml biotinylated antibody and 200 dilution of Avidin-biotinylated peroxidase complex reagent using pig intestine and lymph node as positive tissues and chicken intestine and lung as negative tissues.