

ABSTRACT

ELISA TECHNIQUES FOR THE QUANTITATION OF THE PAN TUMOR MARKER DR-70TM AND THE ANTICOAGULANT HEPARIN

Alicia Lisa Fuentes

The work presented herein has as its focus the application of enzyme-linked immunosorbent assay (ELISA) techniques for the quantitation of the pan tumor marker DR-70TM and the anticoagulant heparin.

The first of these studies involved the screening of the serum of one hundred and forty (140) control patients and eighty-four (84) clinically diagnosed cancer patients, for their DR-70TM levels. The results show that this tumor marker is capable of detecting cervical cancer with a sensitivity of 66.67% at a specificity of 95%. Analysis of the same serum from these patients for their fibrinogen degradation product (FDP) levels using the OncoChekTM assay methodology, show that there is distinct correlation between DR-70TM and OncoChekTM levels. However, OncoChekTM assay prove to be slightly less sensitive than DR-70TM, showing a sensitivity of 50% at a specificity of 95%. The results from both of these assays suggest that DR-70TM levels when used in conjunction with FDP levels can be used as a useful screening method for the early detection of cervical cancer.

A significant finding emerging from the present study is the importance of collecting blood samples in serum separator blood tubes rather than in red top blood tubes, for use in these assays. With respect to DR-70TM levels, significant differences in sensitivity are found for serum collected in serum separator blood tubes (51.9% sensitivity at 96.70% specificity) and serum collected in red top blood tubes (9.52% sensitivity at 94.81% specificity).

Also investigated in this study are the risk factors associated with cervical and ovarian cancers. Significant correlations are found between the DR-70TM and FDP levels and the risk factors such as sexual activity, early age of commencement of sexual activity, number of abortions and old age.

With respect to the quantitation of the anticoagulant heparin, preliminary studies were conducted based on the high affinity of the heparin antidote, protamine sulphate, for heparin. However, due to significant non-specific binding of the peroxidase enzyme conjugate to protamine coated microtitre plates, leading to high background absorbances, the methodology was altered to incorporate instead the heparin antibody, anti-heparin, for coating of the microtitre plates.

The principle of the method developed is based on a competitive ELISA. The optimised conditions were found to be an anti-heparin coating concentration of 8 µg/ml, a biotinylated heparin dilution factor of 25 and a streptavidin-peroxidase dilution factor of 500. Based on these optimised parameters, heparin concentrations in the range of 0.05 – 5 µg/ml can be readily detected.

Keywords: Alicia Lisa Fuentes, DR-70TM, OncoChekTM, Heparin, ELISA.