Thesis Title	Development of Enzyme Immunoassay for Human Chorionic Gonadotropin
Author	Mr. Prakong Sakorn
M.Sc.	Biochemistry
Examining Comittee	Assoc. Prof. Dr. Maitree Suttajit..... Chairman
	Assist.Prof. Dr. Vichai Wongchai..... Member
	Dr. Sukanya Linpisarn..... Member
	Assist.Prof. Pakorn Thaiyanan..... Member

Abstract

The main objective for this thesis work is the development of an enzyme immunoassay (EIA) for human chorionic gonadotropin (HGG). This also includes the preparation of rabbit anti - β - HCG, reference HCG standard and anti - β - HCG- horseradish peroxidase conjugate.

Highly purified HCG was prepared from pooled choriocarcinoma urine by saturated ammonium sulfate precipitation, 80% ethanol precipitation, DEAE Sephadex A.50 and Biogel P-60 chromatography, respectively. The yield and immunologically specific activity of highly purified HCG preparation were 28% and 9250 i.u./mg protein (1st I.R.P for immunoassay). By native - polyacrylamide gel electrophoresis, this HCG preparation showed one major protein band corresponding to that of

patients serum and urine samples.

Moreover, I would like to express my grateful thanks to everyone in my family, expecially my parents for their love, supports, unsderstanding and encouragements. These will be kept in my mind forever.

standard HCG and one minor band with molecular weight less than HCG. β - HCG subunit from the highly purified HCG was obtained by 8 M urea - DEAE Sephadex A.25 chromatography. The yield and immunologically specific activity of highly purified β - HCG subunit preparation were 12% and 14,350 i.u./mg protein, respectively. By SDS - polyacrylamide gel electrophoresis, β - HCG subunit preparation showed only one protein band corresponding to the slower moving band of standard HCG.

Anti - β - HCG was produced in New Zealand white rabbits using highly purified β - HCG subunit preparation as an immunogen and purified by ammonium sulfate precipitation (33% saturation), HCG - Sepharose 4 B and normal human serum protein - Sepharose 4 B affinity chromatography, respectively. The yield and specific activity of highly purified anti-β - HCG preparation were 15% and 1111 Ouchterlony's titer^{-1} /mg protein, respectively. By immunoelectrophoresis, the anti - β - HCG preparation showed no cross - reactivity to normal human serum protein.

Anti - β - HCG was conjugated to horseradish peroxidase by periodate oxidation method. The conjugate was separated by Sephadex G.150 chromatography. About 27% of added horseradish peroxidase was linked to anti - β - HCG.

Highly purified HCG preparation was standardized and used as a reference standard in the development of enzyme immunoassay for HCG by commercial β - HCG EIA. (Hoffman La Roche)

Enzyme immunoassay for HCG base on two - site sandwich system was carried out in polystyrene tubes. Serum sample was incubated with tube - coated with anti - β - HCG. After washing, the tube was added with peroxidase conjugated anti - β - HCG. Finally, peroxidase activity of the conjugate bound to the solid phase was quantified by adding substrate - chromogen mixture (H_2O_2 and O - phenylenediamine), The enzymatic activity was directly proportional to HCG in each sample. A linear calibration curve for HCG concentration ranged between 0-200 mi. u./ml (1^{st} I.R.P. for immunoassay) was obtained with a sensitivity of 4.0 mi.u./ml. For precision study, coefficients of variation (%C.V.) of within assay ranged 5.8 - 8.1% and those of between assay ranged 7.3 - 11.1%. An analytical recovery by dilution study was 101.6 - 111.1% LH and FSH at the concentrations of 100 and 75 mi.u./ml, respectively showed 24 and 15% cross - reactivity in this assay system. Mean serum HCG levels in normal healthy men and women were 5.0 and 9.5 mi.u/ml, respectively. The local - made β - HCG EIA and commercial β - HCG EIA were highly correlated with a coefficient of correlation (r) of 0.945 and a linear - regression equation of Y = 1.01X + 3.34.

In conclusion, the local - made β - HCG EIA is economical and appropriated for the evaluation of serum HCG. It should be useful in clinical diagnosis, monitoring and prognosis of trophoblastic patients, especially hydatidiform mole and choriocarcinoma.