RADIOIMMUNOLOGIC TECHNIQUES USED IN QUANTITATIVE AND QUALITATIVE ANALYSES OF RECOMBINANT HUMAN GROWTH HORMONE (rec-hGH) FOR *E. COLI* CONTAMINANT PROTEINS (ECP) AND hGH-RELATED FORMS DETERMINATION

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We developed different radioimmunologic analytical techniques for the characterization and quality control of rec-hGH.

An immunoradiometric assay (IRMA) was set up, ECP being obtained through an osmotic shock our bacterial strain (RRI) and used for the immunization rabbits.

The antisera so obtained was purified by precipita - tion with caprylic acid followed by affinity chromato - graphy on a column of CNBr-activated sepharose 4B to which the same ECP had been coupled. The purified anti -ECP gama globulins (IgG anti-ECP) were labelled with 125I and used in a sandwich type assay on microtiter plates, adsorbed with the same antibody. Sensitivities of the order of 0.5-0.1 ng/mℓ together with a satisfac- tory inter-assay precision (CV = 4-10%) were obtained. Some of our samples presented levels of ECP < 10 ppm.

Two western blotting techniques for hGH and ECP de- termination were developed to analyze the quality of the rec-hGH.

Samples of rec-hGH eletrophoretically separated on Sodium Dodecyl Sulfate-Polyacrilamide Gels (SDS-PAGE) and transferred to sheets of pure unmodified nitro-cel- lulose were reacted with specific antibody (IGg anti-ECP or IGg anti-hGH) and after subsequent binding of radio iodinated protein A were followed by a radiographic de- tection.

Such techniques, together with RIE are powerful tool to study the different processes of purification steps and for hGH quality control, especially for what con - cerns hGH-related forms, even in impure crude prepara tions.

Data on hGH/ECP antisera cross-reaction and on the presence of dimeric and two-chain hGH clipped forms will be presented and discussed.

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