G - 05

PURIFICATION OF A RECOMBINANT PROTEIN BY HIGH PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY (HPSEC), DIRECTLY FROM BACTERIAL PERIPLASMIC EXTRACTS, FOR IMMUNOASSAY REAGENT PREPARATION.

Ribela, M.T.C.P.; Camargo, I.M.C.; Oliveira, J.E. and Bartolini, P.
Department of Application of Nuclear Techniques in Biological Sciences, IPEN-CNEN/São Paulo, Brazil

A novel, simple and inexpensive procedure for the preparation of a good quality iodination-grade recombinant human growth hormone (rec-hGH), for *in vitro* use, is described.

Rec-hGH with a specific activity of 64% was obtained after a single-step high-performance size-exclusion chromatography (HPSEC) purification of an osmotic shock fluid obtained from a bacterial strain transformed with an hGH expression vector.This preparation was radioiodinated according to conventional techniques (Preparation A). The radioiodinated tracer thus obtained was compared, in terms of quality and applicability, to an analogous preparation, obtained from the same periplasmic extract, after the regular six-step purification process employed for the preparation of clinical grade rec-hGH (Preparation B).

Both tracers presented a similar purification profile, a high radioiodination yield and similar specific bindings at the regular antiserum dilutions used for radioimunoassay (RIA). They also provided the same accuracy, when evaluated with the use of commercial quality control samples in a classical RIA methodology. The storage stability of the two tracers at -20°C consistently showed no significant difference (t-test, $p>0.05$) between their specific and nonspecific bindings over a period of up two months, the decrease in binding being of the order of 0.30%/day.

The novel approach permits the utilization of transformed *E.coli* strains as a source of freshly prepared, radiodination grade recombinant proteins and of other immunoassay reagents such as reference preparations and antigens.