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QUALITY CONTROL IN THE POTENCY DETERMINATION OF hGH EXTRACTS: RADIOIMMUNOASSAY AND RADIO-RECEPTOR ASSAY WITH STANDARDIZED LABELLING AND ASSAY CONDITIONS

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It has been reported by various authors [1–3] that the immunological determination of protein hormones presents severe standardization problems, especially with regard to between-assay and between-laboratory reproducibility. This lack of precision can seriously affect the final accuracy of the determination. In this work we have therefore endeavoured to define a set of experimental conditions that provide at least a reasonable within-laboratory precision when determining hGH extracts by RIA and by RRA.

Since the irreproducibility of the responses seems to be due mainly to the extremely high variability of the interacting reagents (the radiolabelled protein in particular), our goal was to perform assays under conditions of maximum reproducibility in terms of the chemical nature and concentration of the reagents employed. This was achieved by utilizing the following standardization steps:

(1) Setting up an ampoulized, general-purpose, reference preparation of hGH, suitable for radioiodination and use as a secondary standard for RIA and RRA curves. The potency of this preparation was determined against the first

TABLE I. 2 X 2 FACTORIAL ASSAYS – 2nd STD-hGH-IPEN BY THE WEIGHT GAIN OF HYPOPHYSECTOMIZED RATS
(40 rats – 10 days' assay)

Assay No.	Doses (μg/rat)	Potency (IU/mg)	S.D. about dose-resp. line	Combined slope (bc)	Index of precision (λ)	95% confid. limits
1	20–100	3.64	2.63	8.59	0.306	2.19–7.57
2	20–100	3.39	4.60	10.70	0.430	1.59–8.73
3	20–100	4.02	3.51	7.59	0.462	1.97–14.22
4	10–20	4.70	2.10	11.05	0.180	3.29–10.34
				(Statistical treatment: C.I. Bliss)		

TABLE II. LABELLING OF THE 2nd STD-hGH-IPEN WITH THE CHLORAMINE-T METHOD
(one-year study)

Reaction No.	Used radio-activity (μCi)	Relative elution volume of ^{126}I-hGH (V_E/V_0)	Yield of the labelling (%)	Specific activity of the tracer (μCi/μg)	Optimal antibody conc. (%X 10^{-3})	Non-specific binding (%)
1	462.5	2.11	25.7	25.4	0.16	7.2
2	570.0	2.09	23.5	31.1	0.42	6.3
3	883.0	2.15	18.3	32.3	0.63	13.8
4	554.2	2.06	19.5	22.5	0.31	8.7
5	706.2	2.14	23.3	40.7	0.69	6.3
6	616.0	2.03	18.8	25.4	1.04	9.5
7	704.7	2.19	23.6	35.9	0.69	10.4
Mean	642.4	2.11	21.8	30.5	0.56	8.9
S.D.	136.6	0.06	2.9	6.5	0.29	2.7

IRP of hGH for immunoassay 66/217 (WHO) and in a series of 2 × 2 factorial biological assays, performed in hypophysectomized rats against the International Standard of BGH (WHO). Four bioassays, performed throughout a one-year period, provided results and statistical parameters (Table I), in agreement with literature values [4].

(2) Periodically (monthly in our case) labelling a freshly opened ampoule of this same preparation, using the same radioiodination technique and purification process each time, so as to obtain as reproducible specific activities as possible.

(3) Fixing the dilutions of the radiolabelled antigen and of the given ligand (antibody or receptor) preparation in order to maintain them the same throughout the various assays and with the different tracers. Since *the reagent concentrations determine the kinetics of the reaction* these must be maintained constant, unlike other parameters (such as B/F or B/T at 0 dose) which vary with the specific activity of the tracer and, if fixed, will automatically alter antigen and ligand concentrations!

(4) Carrying out the B/F separations via a simple technique, readily reproducible over a long time span with reasonably low unspecific bindings – in our case, via the use of PEG 6000.

(5) Determining the potency of each unknown preparation by reference to a standard curve using the ED_{50} ratio derived from complete assay curves for both standard and unknown.

Although this type of study can be thoroughly evaluated only after analysis of the results and precision (with quality-control samples) over a considerable time period [5], throughout a one-year period we have been able to maintain a reasonable reproducibility in the low specific activity labellings of our reference preparation, as evidenced in Table II.

This procedure gave an interassay coefficient of variation (CV) for the ED_{50} of our RIA standard curves of about 15%, using tracers obtained in five different labellings. Our unknowns and quality-control samples were generally determined with an interassay CV of around 15–20%. However, in the case of RRA, performed using water-solubilized rabbit-liver receptors, the ED_{50} was still found to be highly variable with different tracers and unspecific binding significantly higher than in RIA.

In both types of assay, as well as in the labelling reaction, the isohormone composition of the antigen seems to play a very significant role, a point which is being further investigated.

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MICROLAB 2000, A FREELY PROGRAMMABLE PIPETTING UNIT, INTEGRATED IN RADIOIMMUNOASSAY AUTOMATION

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The Microlab 2000 from Hamilton is a pipetting unit which is freely programmable in the x/y/z axis. It is thus able to be adapted to existing RIA techniques rather than vice versa. In our laboratory we have implemented three different programs: (A) A universal RIA program; (B) a pipetting program; and (C) a diluting program.

In (A) a primary rack with 252 positions (RI) and a secondary rack with 500 positions (RII) are mounted. The Microlab transfers standards and samples from RI to RII with the addition of antiserum and, optionally, label. Simultaneously, the sample can be diluted by a wide range of factors, depending on the size of the mounted syringes and on the sample volume. Serial dilutions of the same sample, as well as different dilutions for different samples, can be performed within the same assay. For the standard curve, only the highest concentrated standard has to be loaded.

Program (B) allows the quick distribution of a chosen volume of a diluent to all tubes (or only parts of them) in either RI or RII.

Program (C) allows, when a dilution factor and an end volume are given, the dilution of samples from one rack into another.

RII is built with racks from a LKB-γ-Counter "Rackgamma". These are mounted with inserts (Type No. 1270–138), which hold reaction vials (Type No. 72.708) from Sarstedt rigidly. After the centrifugation of 30 racks at one step, the racks can be decanted and put in the only slightly modified "Rackgamma". We have, therefore, built up a system where, from the first to the last step, the tubes are never handled. There is no way to lose identification of the samples and we would, therefore, designate our system a true RIA automate.