SETTING UP A LATIN AMERICAN REFERENCE PREPARATION OF HUMAN THYROTROPIN AND ITS VALIDATION THROUGH AN INTERNATIONAL INTER-LABORATORY STUDY*

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Abstract

SETTING UP A LATIN AMERICAN REFERENCE PREPARATION OF HUMAN THYROTROPIN AND ITS VALIDATION THROUGH AN INTERNATIONAL INTER-LABORATORY STUDY.

A human thyrotropin (hTSH) extract to be used within the IAEA-organized ARCAL VIII programme (Regional Co-operative Arrangements for Promotion of Nuclear Science and Technology in Latin America), whose main objective is the preparation and standardization of reagents and assays for the determination of thyroid related hormones, has been prepared in the laboratory of the National Nuclear Energy Commission (IPEN-CNEN), São Paulo. The purified and ampouled reference preparation was first submitted to an eight-month preliminary intra-laboratory quality control. Its potency, determined via immunoradiometric assay (IRMA) ($n = 6$ assays) against NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA) and NETRIA (North East Thames Region Immunoassay Unit, London, United Kingdom) reference preparations, was 58.8 ± 6.6 μIU/ampoule, while ampoule variability showed an intra-assay inter-ampoule ($n = 6$) coefficient of variation of 4.1%. After testing this preparation in an ARCAL VIII Regional Training Course, a certain number of ampoules were distributed to each one of the 14 participating Latin American countries, to six more ARCAL VIII participating Brazilian laboratories and to the WHO Reference Laboratory (Hammersmith Hospital, London, United Kingdom). A total of 185 unknown samples were analysed by 14 different laboratories using the Latin American and NETRIA hTSH reference preparations. A linear regression analysis carried out on these data presented a correlation coefficient $r = 0.954$ and a slope $= 1.03$, with a highly significant correlation ($p < 0.001$) between the two sets of results. The stability of the Latin American Reference Preparation was also confirmed through a 20 month intra-laboratory study. The WHO Reference Laboratory found it sufficiently pure, the standard curve running parallel to a well-known commercial preparation which is calibrated against WHO-IRP-hTSH-80/558.

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1. INTRODUCTION

Worldwide, human thyrotropin (hTSH) assay is the most widely used technique in neonatal hypothyroidism screening programmes, an urgent necessity for most Latin American countries [1]. Thanks to the experience already acquired by our laboratory in pituitary hormone extraction, purification and control [2] we could offer our collaboration for the setting up and distribution of a Latin American reference preparation of hTSH. In this work we closely followed the NETRIA (North East Thames Region Immunoassay Unit, London, United Kingdom) programme [3] and the recommendations and publications of the National Institute for Biological Standards and Controls (NIBSC) (London, United Kingdom), a World Health Organization (WHO) laboratory for standard preparations [4].

An intra-laboratory study, directed to a first quality control of the ampouled reference preparation, was carried out at the National Nuclear Energy Commission (Regional Co-operative Arrangements for Promotion of Nuclear Science and Technology in Latin America) (IPEN-CNEN), São Paulo, the Brazilian ARCAL VIII reference laboratory. The international interlaboratory work was organized considering all the fourteen ARCAL VIII participating countries and the WHO Collaborating Centre for Immunoassay. The main purpose was the validation of this Latin American reference preparation of hTSH through collaborative work designed not only to lead to the local production of this and other imported reagents, but also to a useful interchange of scientific information and technology.

2. METHODS

2.1. hTSH extraction

Extraction, purification and 'in bulk' controls of pituitary hTSH are described in Paper IAEA-SM-324/61.

2.2. Preparation of the ampoules

The hTSH preparation was adequately diluted and, after addition of 2 mg/mL of RIA-grade bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) and 10 mg/mL lactose, was distributed in ampoules (1 mL each). After 48 hours' lyophilization and 4 days' secondary desiccation under vacuum in the presence of phosphorus pentoxide, the ampoules were sealed by heat fusion, under nitrogen, and stored at 4°C. After reconstitution with 1 mL of distilled water, six ampoules were used for the determination of inter-ampoule variation, while the hTSH content (or potency) was determined in an immunoradiometric assay (IRMA) design (NETRIA), through $n = 6$ independent assays. Each assay determined the mean of seven serial

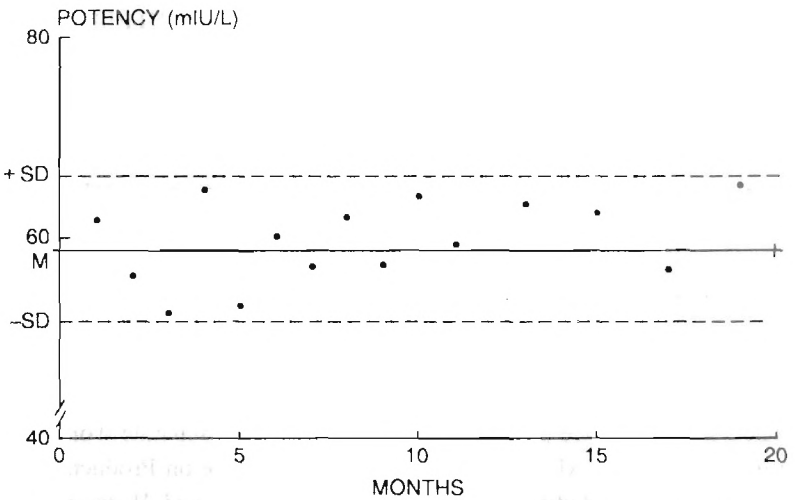

FIG. 1. Stability of hTSH-IPEN ampouled reference preparation, determined over a 20 month period.

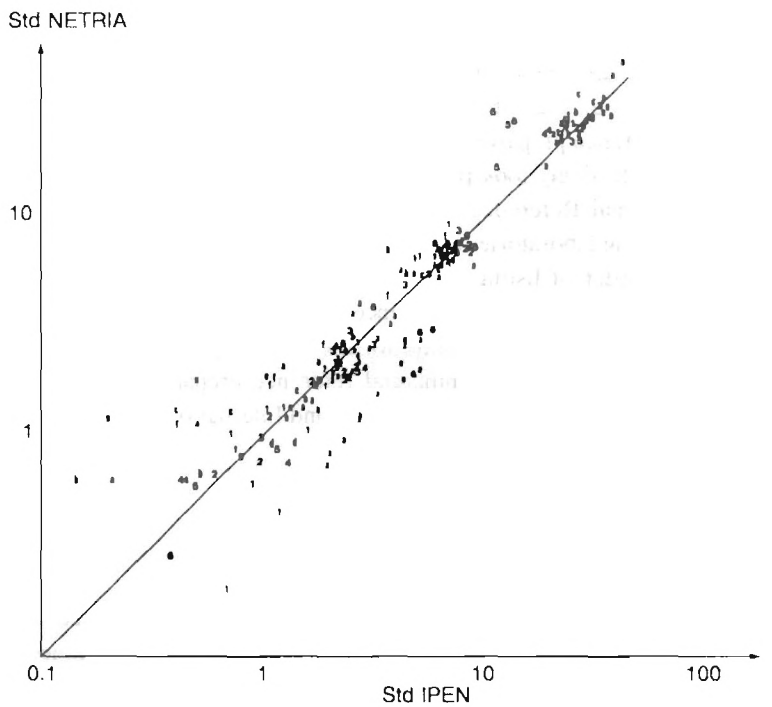

FIG. 2. Results of an inter-laboratory comparison between hTSH-NETRIA (United Kingdom) and hTSH-IPEN (Brazil) reference preparations on n = 185 unknown samples from: (a)–(f) different laboratories from the ARCAL VIII Latin American countries; (1)–(6) Brazilian ARCAL VIII participating laboratories.

dilutions covering the whole curve range, against the same NETRIA-hTSH reference preparation and NIDDK-hTSH-RP-1 (kindly donated by the National Hormone and Pituitary Program, Baltimore, MD, USA). NETRIA and NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA) reference preparations had both been calibrated against the second international reference preparation IRP-hTSH-80/558 for immunoassay (NIBSC-WHO, London). The stability of the preparation was checked over a 20 month period (Fig. 1), determining its hTSH IRMA content by opening a new ampoule each month for the first year and every two months in the following period.

2.3. Collaborative study

The hTSH reference preparation was tested by four separate working groups during the Second ARCAL VIII Regional Training Course on Production and Use of Bulk Reagents for Radioimmunoassay of Thyroid Related Hormones, held in São Paulo, Brazil, from 6 to 24 November 1989. Ten ampoules were distributed to each representative of the 14 ARCAL VIII participating countries, while six ampoules were given to each one of the six additional ARCAL VIII participating Brazilian laboratories. They were all asked to estimate the hTSH content of local quality control and unknown serum samples, in different ranges of the standard curves, in terms of our and NETRIA reference preparations. A calculation of the potency of our reference preparation was also requested. Only fourteen laboratories in nine countries effectively took part in this study; these are listed in Fig. 2. Each ARCAL VIII National Reference Laboratory is represented by a letter while the Brazilian participating laboratories are indicated by a number. The sequence does not coincide with the order of listing.

Some ampoules of the hTSH reference preparation were also kindly analysed by Dr. Sufi (WHO Collaborating Centre for Immunoassay, Hammersmith Hospital, London) in comparison with a commercial reference preparation (Serono MAIA[1] clone kit) which is calibrated in terms of the second International Reference Preparation, IRP-hTSH-80/558.

3. RESULTS AND DISCUSSION

The Latin American reference preparation of hTSH presented an IRMA activity of 58.8 ± 6.6 μIU/ampoule (interassay CV = 11.2%) determined in intra-laboratory comparison (In Fig. 2 of Paper IAEA-SM-324/61 an example of this determination is presented). The mean of the potency estimate determined in the collaborative study (n = 19 assays) was 60.5 ± 10.2 μIU/ampoule (inter-laboratory

[1] MAIA = Monoclonal anti-IgG assay.

CV = 16.9%). The inter-ampoule (n = 6) intra-assay coefficient of variation was 4.1%. The preparation also presented a satisfactory stability over the 20 month period analysed, as shown in Fig. 1. Its purity and equivalence to the Serono MAIA clone kit standard curve were also declared by the above mentioned WHO Collaborating Centre.

A highly significant correlation ($p < 0.001$) with a correlation coefficient $r = 0.954$ and practically no bias (slope = 1.03) was found in the determination of 185 unknown samples carried out comparing the Latin American and NETRIA reference preparations.

In Fig. 2 the results of this international collaborative study are presented, with the distribution of the values around the identity curve. We can observe higher scatter in the lower dose region.

We conclude that this Latin American reference preparation can be usefully applied in substitution for the imported reagent. A large number of ampoules have been prepared in our laboratory and are under testing for more rational and useful distribution, giving continuity to our work.

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