

Gonadotropin Standards – Achievements and challenges for the future

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A major principle that has underpinned the World Health Organization (WHO) Biological Standardisation Program for more than eighty years has been the premise that for heterogeneous biological analytes such as proteins and glycoproteins International Standards (IS) to which measurement procedures can be referenced are essential. Early IS originally established for use in bioassays and with assigned units reflecting biological function were often also adopted for this purpose by those developing immunoassays in the 1960s and 1970s. Consequently, for many hormone analytes including LH, FSH and hCG, the use of International Units when reporting clinical results of immunoassays continues.

WHO international biological standards are generally prepared in single batches, usually of 4000 ampoules, contain milligram quantities of stabilizing excipients (usually sugars and/or carrier proteins), and undergo rigorous accelerated degradation studies to ensure that they will remain stable for decades. Potency has usually been assigned in International Units following an International Collaborative Study, using a consensus estimate.

Despite significant improvements in both immunoassay technology and in methods for protein purification and characterization, controversies identified at a meeting ten years ago¹ - including how International Standards should best be replaced, whether use of material from recombinant rather than natural sources is desirable, whether use of molar units is feasible, and how and whether appropriate standards can be prepared for variably heterogeneous materials - remain relevant today, particularly for the gonadotropins. With increasing demands for International Standards, the traditional batch size may now last no longer than a decade, requiring replacement that can be both problematic and time-consuming. For example, adoption of any of three available candidate replacement standards – one pituitary-derived and two

recombinant - for the current FSH International Standard (Reference Preparation 94/632) would probably worsen between-method agreement and in one case would cause a three-fold decrease in FSH concentrations as measured by immunoassay, with major implications for clinical practice.² While recombinant DNA technology is increasingly likely to be the method of choice when preparing candidate reference materials in the future, use of such preparations may cause difficulties with assay validation, as has already been demonstrated for TSH. Such difficulties reflect the heterogeneity inevitably observed for complex biological analytes such as the gonadotropins, to which post-translational modification, the presence of degradation products and circulating subunits may all contribute. Establishing reference standards calibrated in molar units and enabling unambiguous description of the species or isoforms recognized in different assay systems (i.e. what methods are measuring) represents a major step forward and is an approach for which hCG provides a prototype model.

1. Bristow AF. Standardisation of protein hormone immunoassays: current controversies. *Proc UK NEQAS Mtg* 1998;**3**:66-73.
2. Sturgeon CM, Ellis AR. Standardization of FSH, LH and hCG – Current position and future prospects. *Mol Cell Endocrinol* 2007; **260-262**:301-9.