

## Standardisation of immunoassays for FSH, LH and hCG: Current position and future prospects.

Catharine Sturgeon and Andy Ellis

UK NEQAS for Peptide Hormones, Department of Clinical Biochemistry, Royal Infirmary, Edinburgh EH16 4SA, UK.

Data from proficiency testing schemes demonstrate some improvement in overall between-laboratory agreement for serum gonadotropin assays over the last ten years. However geometric coefficients of variation are still >10%, and there is clearly scope for improvements that would potentially benefit patient care. Factors contributing to the differences observed include errors in calibration and differences in choice of antibodies and assay design. Of these, errors of calibration are most readily addressed, the others ideally requiring knowledge both of what present assays are measuring and of what it is clinically desirable to measure. Here we review the International Reference Preparations (IRP) currently available for FSH, LH and hCG and consider prospects for future improvement.

**FSH.** Commercially available immunoassays are standardised against IRP 78/549 (no longer available) or interim RP 94/632, both of which were prepared from the same partially purified pituitary extract. While results for serum pools vary between methods by up to 30%, most methods show reasonably accurate recovery (94-115%) of added standard. Three candidate replacements for the impure pituitary preparation are available. International Standard (IS) 83/575 is a highly purified pituitary preparation containing a high proportion of acidic isoforms. Acidic isoforms are more potent in *in vivo* bioassay than neutral isoforms, and consequently introduction of IS 83/575 as a standard for immunoassay would cause a three-fold decrease in observed FSH concentrations. There are also two recombinant preparations: IS 92/510 for immunoassay and IS 92/642 for bioassay. Evidence suggests that use of either as an immunoassay standard would be likely to worsen between-method agreement.

**LH.** Commercially available immunoassays are calibrated against the highly purified pituitary extracts IS 80/552 or IRP 68/40 (no longer available). Results on serum pools vary between methods by up to 30%. Many methods show inaccurate recovery (85% - 180%) of added standard, indicating considerable scope for improvement. No candidate replacement exists, although a recombinant preparation, IS 96/602, is available as a standard for bioassay. Its activity was defined to maintain continuity with the urinary LH standard IS 98/704, so it is unlikely to be suitable as a standard for immunoassay.

**hCG.** Commercially available immunoassays are calibrated against IS 75/537 or IS 75/589, which are essentially equivalent. Most methods show near quantitative recovery but several over-recover significantly (>115%). Highly purified International Reference Reagents for six important hCG-related molecules [intact hCG, nicked intact hCG (hCG<sub>n</sub>), hCG beta-subunit (hCG<sub>β</sub>), nicked hCG beta-subunit (hCG<sub>βn</sub>), hCG alpha-subunit (hCG<sub>α</sub>) and hCG beta-core fragment (hCG<sub>βcf</sub>)], prepared under the auspices of the IFCC, are also available.<sup>1</sup> The first hCG-related reference reagents to be calibrated in molar (rather than arbitrary) units, their primary purpose initially is to assist manufacturers and users alike in characterizing what their hCG assays are measuring, work that is currently in progress.

Proficiency testing data indicate that there is little relationship between method bias and recoveries for these three analytes, suggesting that while correct calibration is essential for improved method comparability, antibody specificity and assay design are also important. An hCG antibody-mapping project has made it possible to make some broad recommendations regarding antibody combinations that should yield the hCG assay specificities most appropriate for particular clinical applications.<sup>2</sup> A separate study has highlighted, also for hCG, the importance of the choice of secondary standard preparations (i.e. the "kit calibrators").<sup>3</sup> Heeding the results of such studies, as well as paying careful attention to accurate calibration, when designing the next generation of hCG assays - and extending this approach to LH and FSH - should lead to improved standardisation and diagnostic reliability of these clinically important assays.

<sup>1</sup> Bristow A, Berger P, Bidart J-M, Birken S, Normal R, Stenman U-H, *et al.* Establishment, Value Assignment, and Characterization of New WHO Reference Reagents for Six Molecular Forms of Human Chorionic Gonadotropin. *Clin. Chem* 2005; **51**: 177-182.

<sup>2</sup> Berger P, Sturgeon CM, Bidart JM, Paus E, Gerth R, Niang M, *et al.* The ISOBM TD-7 Workshop on hCG and Related Molecules. Towards user-orientated standardization of pregnancy- and tumor marker diagnosis: Assignment of epitopes to the three-dimensional structure of diagnostically and commercially relevant monoclonal antibodies (mAbs) directed against human Chorionic Gonadotropin (hCG) and derivatives. *Tumor Biology* 2002; **23**: 1-38

<sup>3</sup> Cole LA, Sutton JM, Higgins TN, Cembrowski GS. Between-method variation in human chorionic gonadotropin test results. *Clin Chem* 2004; **50**: 874-882