

Development of Reference Methods and Materials for the Determination of Hormones in Human Serum

Many life functions are regulated by hormones. When hormone levels deviate from normal, serious health consequences may result. Timely and effective treatments require accurate diagnoses of hormone levels. New reference methods and reference materials are being developed to support accuracy and traceability of clinical laboratory measurements of non-steroid hormones related to thyroid function and other metabolic processes

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NIST is developing reference methods and Standard Reference Materials (SRMs) to support accuracy and traceability for hormone assays. New methods, based on liquid chromatography/mass spectrometry (LC/MS), have been developed for estradiol-17 β [1], adding to the suite of reference methods previously developed for other hormones including cortisol [2], thyroxine (T4) [3], and triiodothyronine (T3) [4]. Method development is underway for progesterone and testosterone. These methods will be applied to the certification of a new hormones-in-human-serum SRM (SRM 971). For a Consultative Committee for Amount of Substance – Metrology in Chemistry (CCQM) Pilot Study, a new LC/MS-based method was developed for measuring 19-norandrosterone in urine [5]. This compound is the principal urinary metabolite of nandrolone and some other synthetic testosterone substitutes.

Improving the accuracy of clinical assays for hormones will improve diagnoses and result in earlier treatments. The new methods and the SRM will help improve accuracy of these assays and will also provide high-order reference systems for traceability.

Reference method development has been completed and the method published for estradiol-17 β , the most potent estrogen hormone. This method is based on isotope dilution LC/MS. Tandem mass spectrometry (MS/MS) is used to provide greater specificity in the measurements. Because the concentration of estradiol-17 β is very low in blood, it was necessary to derivatize the compound with dansyl chloride to enhance its sensitivity in the mass spectrometer. This method, along with those developed for cortisol, T4, and T3, and those to be developed for testosterone and progesterone, will be applied to the certification SRM 971. This new SRM consists of two pools, one from

normal adult males and one from normal, premenopausal adult females.

The urinary metabolite 19-norandrosterone may be naturally present at very low levels in urine, but is elevated when certain widely used, but banned, testosterone-like steroids such as nandrolone are used. Methods based upon gas chromatography/mass spectrometry (GC/MS) have been used to detect this metabolite. Recently, the National Measurement Laboratory in Australia organized a pilot study through the CCQM on measurement of this metabolite in urine. To participate, NIST needed to develop a method and investigated both LC/MS and GC/MS approaches. The LC/MS approach proved to be more sensitive and more reproducible, so a method based on LC/MS/MS was developed and thoroughly tested [5].

Future Plans:

Method development will continue and new SRMs developed as needed. The need for an SRM focused on metabolites of testosterone-like substances will be investigated.

Publications:

1. Tai, S. S-C. and Welch, M.J. "Development and Evaluation of a Reference Measurement Procedure for the Determination of Estradiol-17 β in Human Serum ID-LCMS and LC-Tandem MS," *Anal. Chem.* 77, 6359-6363 (2005).
2. Tai, S. S-C. and Welch, M.J. "Development and Evaluation of a Candidate Reference Method for the Determination of Total Cortisol in Human Serum Using Serum ID-LCMS and LC-Tandem MS," *Anal. Chem.* 76, 1008-1014 (2004).
3. Tai, S. S. C., Sniegowski, L. T., and Welch, M. J. "Determination of Thyroxine in Human Serum ID-LCMS with Electrospray Ionization," *Clin. Chem.* 48, 637-642 (2002).
4. Tai, S.S-C., Bunk, D.M., White V, E., Welch, M.J. "Development and Evaluation of a Candidate Reference Method for the Determination of Total 3,3',5-Triiodothyronine in Human Serum ID-LC -Tandem MS," *Anal. Chem.* 76, 5092-5096 (2004).
5. Tai, S. S-C., Xu, B., Sniegowski, L.T., and Welch, M.J. "Development and Evaluation of a Candidate Reference Measurement Procedure for the Determination of 19-Norandrosterone in Human Urine Using Isotope-Dilution LCMS and LC-Tandem MS," *Anal Chem* 2006, 78, 3393-3398.