

Project Review

“Development of Ultrasensitive DNA Aptamer-Based Assays for Natural and Recombinant Human Growth Hormone in Serum and Urine”

J. Bruno (Operational Technologies Corporation, San Antonio, Texas, USA)

The primary problems associated with routine detection of illegal doping with recombinant human growth hormone (rhGH) have been the identical amino acid sequences of natural and rhGH and the extremely low levels of hGH in urine (0.1% to 1% of serum levels).

Operational Technologies Corp. (OpTech) proposes to develop DNA aptamer-based assays capable of detecting and discriminating:

- 1) natural hGH,
- 2) 20kD, 22kD, and heavier glycosylated hGH isoforms, and
- 3) E. coli-modified rhGH “markers” which exist as ~ 2% “contaminants” of the total hGH in recombinant hormone preparations (Hepner et al., 2005).

The development of aptamer reagents for detection and discrimination of hGH and rhGH or its E. coli-modified forms is novel and carries certain advantages vs. traditional immunoassays including potentially improved sensitivity and specificity, reduced cost (due to obviating of animal hosts and expensive antibody production and purification procedures), and an assured supply of identical reagent from lot-to-lot once the aptamer DNA sequences are identified.

In addition to the potential advantages of DNA aptamers over antibodies, OpTech intends to couple its aptamers to tosyl-magnetic beads (MBs) to concentrate the hGH-associated analytes in serum or urine and use either quantum dots (QDs) or electrochemiluminescence (ECL) to produce assays with lower than femtogram detection limits. The combination of aptamers, MBs, and QDs or ECL may produce assays capable of detecting the ~ 2% modified marker forms of rhGH in urine to eliminate invasive blood draws and facilitate frequent testing of urine. If successful, the assays may even be performed on-site at sporting events with a handheld fluorometer such as the PicoFluor™ or a portable ECL sensor. The preferred embodiment will use OpTech’s one step (homogeneous) aptamer plastic-adherent cuvette assay and handheld reader as shown on OpTech’s spin out biotech company’s website (www.pronucleotein.com) and recently published (Bruno JG, et al. J. Fluorescence 2008).