

## **Urgent Need for a Hyperglycosylated hCG (H-hCG) Standard**

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**The Problem:** Hyperglycosylated hCG (H-hCG) is a large hCG variant with extra-large sugar side chains produced by cytotrophoblast cells in early pregnancy, choriocarcinoma and testicular and other germ cell malignancies. H-hCG accounts for  $96 \pm 23\%$  of total hCG in the 3rd complete week of gestation (3 weeks 0 - 6 days),  $68 \pm 9\%$  in the 4th,  $51 \pm 4.0\%$  in the 5th, and  $25 \pm 4.5\%$  in the 6th complete week. Proportions decline rapidly thereafter (Cole LA, et al., *Clin Biochem*, 2003). Similar proportions are observed in early pregnancy serum and urine samples. H-hCG is the principal hCG-related molecule present in early pregnancy urine samples making it the ideal molecule for pregnancy detection. Of 14 common serum laboratory hCG tests, two, Serono MAIAclone & Dade Dimension, poorly detected or failed to detect H-hCG. Ten tests gave very low results when testing serum supplemented with H-hCG. Only the DPC Immulite detected absorbance-calibrated (278nm) pure hCG (100%) and pure H-hCG (99%) near-equally. We similarly evaluated 4 point-of-care urine tests and all had 2-fold lower sensitivity for H-hCG than for hCG. Of 18 brands of home pregnancy tests examined, 11 poorly detected H-hCG, with 2- to 8-fold lower sensitivity. Calling these tests "pregnancy tests" is in some respects a misnomer. False negative pregnancy test lead to women missing that they are pregnant, to late discovery, and thus drinking and use of inappropriate drugs.

How has this happened? Why are few pregnancy test optimized for early pregnancy detection. While WHO and other hCG standards are widely obtainable, there is no available standard for manufacturers to optimize test to H-HCG, the principal molecule in serum and urine in early pregnancy.

Why are there available standards for hCG, nicked hCG, hCG free  $\beta$ -subunit, nicked hCG free  $\beta$ -subunit and hCG  $\beta$ -core fragment, but not for H-hCG, seemingly, the most important variant of hCG? hCG can be extracted directly from 8-12 weeks pregnancy urine, time of peak hCG production, or made by recombinant technology in CHO cells. H-hCG is made at relatively low concentrations (though high % of immunoreactivity) in early pregnancy (3-6 weeks gestation). Levels are too low for efficient extraction. It is not made by CHO-cells. Choriocarcinoma and testicular cancer patient urine contains high concentrations of H-hCG, but a highly nicked variant, that is very unstable. As such, we have no simple source, no obvious standard, and so assay go uncalibrated.

**Addressing the problem:** As published (Cole LA, et al., *Clin Biochem*, 2003), choriocarcinoma cell lines produce a non-nicked H-hCG. While JEG-3 cell line produces solely H-hCG, Jar and BeWo produce combinations of H-hCG, H-hCG free  $\beta$  and other hCG carbohydrate variants. We know of 2 companies, desperate to make a viable standard, that have grown JEG-3 cell in large culture chambers or fermenters. They have produced hundreds of milligrams this way. This is seemingly the method for making an urgently needed international standard.