Technical Brief

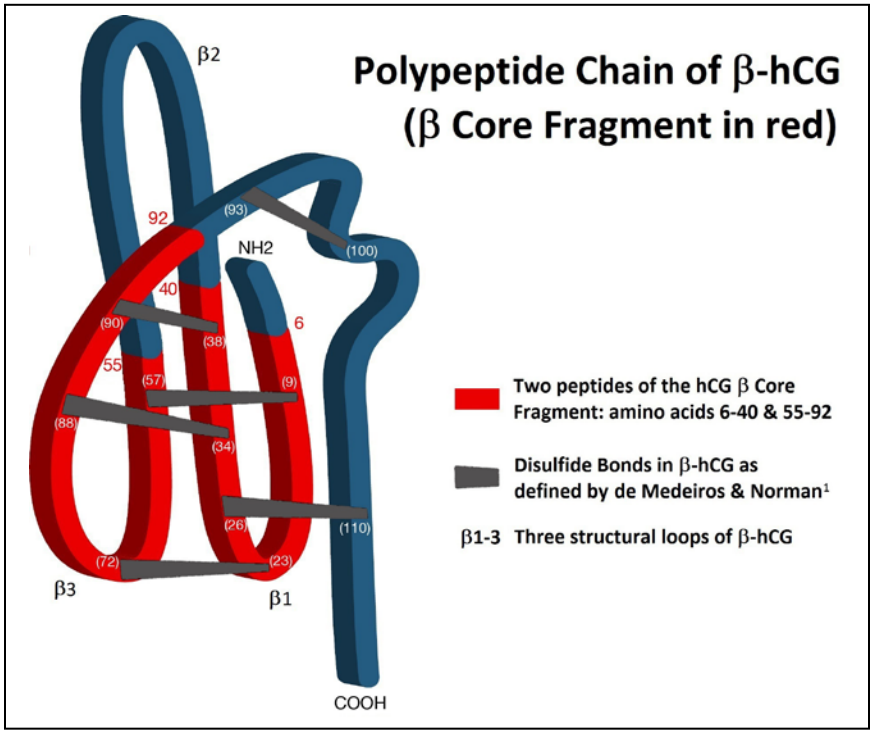
hCG β Core Fragment Interference in Total hCG Assays

Commercial immunoassays for total hCG in serum and urine are not detecting a significant portion of the hCG β Core Fragment, thereby under-reporting the total amount of hCG present in patient samples.

The measurement of serum or urine levels of total Human Chorionic Gonadotropin (hCG) has long been used for pregnancy detection and identifying conditions such as choriocarcinoma of the uterus, ovarian cancer, testicular cancer, miscarriage, ectopic pregnancy, Down Syndrome, and more.[1] While there are many commercially available assays for total hCG, evidence is mounting rapidly that these assays do not accurately report the total amount of hCG present in the test sample.

The hCG molecule has a molecular weight of 38,000 Daltons, contains 237 amino acids, and is composed of two subunits, designated alpha (α-hCG) and beta (β-hCG). After intracellular synthesis and assembly, hCG is secreted as a bioactive heterodimer and is ultimately metabolized by the liver, ovaries, and kidneys, resulting in a number of different molecular forms, the majority of which are excreted in the urine.

There are a multitude of hCG forms found in serum and urine, the most studied of which include intact hCG, hyperglycosylated hCG, nicked hCG, nicked hCG without the C-terminal peptide, asialo hCG (missing sialyl groups or sialic acid), α-hCG, β-hCG, nicked β-hCG, and hCG β core fragment (hCGβcf).[1,2,3,4] Of these variants, the National Institute for Biological Standards and Control (NIBSC) and the World Health Organization (WHO) have identified six as the most relevant and offers reference preparations for each. *(See table below.)*



WHO Standards for hCG Variants

Molecular Variant	Abbreviation	NIBSC Reference ID
Intact hCG	hCG	WHO IRR 99/688
Nicked hCG	hCGn	WHO IRR 99/642
hCG, α Subunit	α-hCG	WHO IRR 99/720
hCG β Subunit	β-hCG	WHO IRR 99/650
Nicked hCG β Subunit	β-hCGn	WHO IRR 99/692
hCG β Core Fragment	hCGβcf	WHO IRR 99/708

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Immunoassays for hCG, regardless of format (ELISA, lateral-flow, etc.), should identify all the molecular variants, providing an accurate assessment of the total amount of hCG present in a patient sample. This, however, is not the case, as several recent studies indicate that one variant, hCGβcf, is responsible for the widespread under-reporting of total hCG values.

hCGβcf is made by the removal of the β2 loop of β-hCG, 5 N-terminal amino acids, and amino acids 93-145 of the C-terminal end. *(See figure above.)* Although it is highly immunogenic, the biological function of hCGβcf, if any, is

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unknown at present.[1] When hCG is metabolized and excreted from the body, however, it is hCGβcf that represents the largest molecular variant of hCG in urine. As such, the omission of hCGβcf levels in total hCG determinations is potentially problematic.

The ability of commercially available total hCG tests to accurately detect hCGβcf is poor, as most commercial assays report falsely low results, due to hCGβcf interference.[2,3,4,5,6,7,8]

In a 2014 study, Nerenz et al. evaluated several point-of-care hCG devices that provide qualitative results, measuring each test's ability to accurately measure hCGβcf.[5] Of the 11 kits studied, 9 recorded significant false-negative results due to misrepresentation of hCGβcf levels.

Similar results were found earlier by Cole et al., who evaluated 12 different automated total hCG assays for their ability to recognize 9 different hCG variants:

- intact hCG,
- hyperglycosylated hCG,
- nicked hCG,
- nicked hCG missing the C-terminal peptide,
- nicked hyperglycosylated hCG,
- asialo hCG (missing sialyl groups or sialic acid),
- β-hCG, and
- hCGβcf.[2]

Of the 12 assays, not a single test recognized all 9 hCG variants. One kit did recognize 8 of the 9 variants, but only 4 detected 4 of 9 hCG forms, 2 recognized 3 of 9 forms, another 4 detected 2 of 9 forms, and 1 kit detected only 1 of the 9 hCG forms.

In another study, Whittington et al. reviewed 8 hCG assays for their ability to detect 5 of the 6 WHO International Reference Reagents (IRR): intact hCG, nicked hCG, β-hCG, nicked β-hCG, and hCGβcf.[4] Each IRR was spiked into normal, hCG-free, human serum, then assayed. All 8 assays accurately detected intact hCG and nicked hCG, while 7 of the 8 accurately reported the β-hCG and nicked β-hCG levels. The detection of hCGβcf was, however, much less than desired as only 2 of the 8 assays detected it at all, and both of those appreciably under-reported the actual levels.

False-negative hCG tests due to the presence of hCGβcf may have dire consequences. Many trophoblastic and non-trophoblastic tumors produce β-hCG, and highly elevated levels of β-hCG associate strongly with a poor prognosis for these patients.[8,9] Falsely low hCG measurements could delay diagnosis and treatment, thereby contributing significantly to increased mortality. In addition, false-negative pregnancy tests at home may result in delays in prenatal care and in the continuation of behavior that may put the fetus at risk.

It is important to note that the false-negative effect hCGβcf has on total hCG measurements has been reported to the US FDA, but no action has been taken to date.[7] Nevertheless, it is anticipated within the clinical diagnostic industry that testing for hCGβcf will soon be a requisite protocol for the release of a total hCG assay.

References

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