PROJECT REVIEW

“Isoform specific tests to detect GH doping”

M. Ueki, T. Otsuki, H. Tanaka, K. Aoki (Japan Chemical Analysis Center, Japan), **T. Kawahara** (Japan Institute of Sports Sciences, Japan) **M. Irie**, (Toho University, Japan)

We have developed immunoassays for 20K/22KGH isoforms in serum, and the detectability of GH doping was proven.

This proposal is aiming to make the ELISA kits available for the antidoping laboratories, to extend the methods to detect GH in urine samples, and to maximize the assay performance by multiplexing using flow cytometry that is common to the homologous blood transfusion test.

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Result and Conclusion;

It is well known that active ingredient of pharmaceutical hGH preparation is 22KDa-hGH isoform (22KGH) and administration of GH increases 22KGH to 20KDa-hGH (20KGH) isoform ratio. Because production of those isoforms is being regulated genetically and formed as a result of splicing mRNA during expression of hGH-N gene, a basal 22KGH/20KGH level in human is very stable. We have established highly sensitive sandwich immunoassays for 22KGH and 20KGH in serum, the assay performance was validated according to WADA ISL and the methods have been implemented on 3 assay platforms, i.e., 96-hole microplate assays, multiplexed 20KGH&22KGH microsphere immunoassay for Luminex® and multiplexed immunoassay by flowcytometry.

For all three of the assay platforms, the performance is confirmed to be basically same, and the LOQ for 22KGH and 20KGH are 10 and 20pg/mL serum, respectively. Essentially no age-related decrease, no sex-, ethnical- and sporting type-dependent differences of 22KGH/20KGH are observed. Advantage of the detection of GH doping by monitoring the isoform ratio would be less factors influencing the test results, simplicity of the marker to be monitored, namely, two single molecules that are well characterized in the literatures. Serum isoform ratio immediately goes up from about 10 to several thousand after single GH dose, and returned to the base level in about 32 hours. Our data represented possibility to extend the isoform selective GH test to urine samples.