Immunocapture combined with tandem-MS detection for differentiation between hCG isoforms in clinical relevant samples

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INTRODUCTION

hCG is not a single molecule although it is often referred to as one. The total hCG response that is generated from the analysis of a clinical sample has to represent the sum of all the different hCG variants produced by the biological process in progress, whether a healthy pregnancy or malignancy. Whereas numerous immunoassays have been developed to ensure detection of the entire spectrum of isoforms displayed by the hCG molecule, significant variation has been demonstrated in how these isoforms are recognized by the antibodies in different immunoassays. The aim of this study was to establish a method using the dual selectivity of the immuno-extraction combined with the mass spectrometry detection for the differentiation between various hCG isoforms in clinically relevant samples.

METHODS

The monoclonal antibody E27 was immobilized on magnetic beads, and used for the extraction of hCG variants spiked to both urine and serum. The captured hCG variants were subjected to tryptic digestion, generating peptides that were further separated by liquid chromatography and detected by mass spectrometry (MS). The detection of the generated signature peptides was performed in the selected reaction monitoring (SRM) mode using a triple quadrupole detector.

RESULTS

This immuno-MS method demonstrated the extraction and detection of different hCG variants from both spiked serum and urine samples, but also from cancer patient serum samples and urine samples of pregnant women. Additionally the method enabled the distinction between the following hCG variants present in the samples: unmodified hCG beta subunit, nicked hCG beta subunit and hCG beta core-fragment.

CONCLUSION

We conclude that the orthogonal selectivity conferred by the combination of immunoaffinity extraction and LC-MS/MS analysis offers valuable complementary information to the conventional immunoassays.