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Response to Letter to the Editor regarding “Development of an antibody-free ID-LC MS method for the quantification of procalcitonin in human serum at sub-microgram per liter level using a peptide-based calibration”

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Dear Editor,

In this issue, Tölke et al. responded to our recently published paper describing an antibody-free method for procalcitonin (PCT) quantification by isotope-dilution coupled to liquid chromatography and tandem mass spectrometry (ID-LC-MS/MS) [1]. This group proposes a MS-based quantification strategy that relies on polyclonal antibody-based enrichment for PCT affinity purification at the protein level [yet unpublished].

We agree with Tölke et al. that immunoaffinity has several advantages, especially a less laborious sample preparation step than our two-step solid-phase extraction (SPE) at protein and peptide levels. Although SPE is presumably a less specific sample preparation procedure, we believe that this turns into an advantage in the sense that our antibody-free method is more prone to quantify the total PCT content, particularly if PCT is bound to other proteins or if the different forms of PCT are not recognized by the antibody with the same affinity. Additionally, we believe that antibody-based capture of

PCT, mainly when using polyclonal antibodies, can be subject to a higher risk of batch-to-batch variations in specificity that could compromise the method's intermediate precision over a prolonged time [2, 3]. Moreover, polyclonal antibodies can be subject to cross-reactivity issues that could result in the extraction of other proteins than PCT. Although the method's specificity is probably not affected thanks to the MS-based detection, this could potentially reduce the method's sensitivity. Even if studies are currently addressing this limitation [4], our work aimed to avoid this effect by developing an antibody-free procedure.

Another significant difference is that Tölke's method relies on protein-based calibrators while ours involves peptide-based calibrators. As described in our paper, we agree that protein-based calibration relying on a non-labelled recombinant protein as primary calibrator and a labelled recombinant protein as internal standard is preferable because the internal standard is added from the start of the sample preparation procedure, thereby compensating variability and material losses occurring during the whole procedure. Also, a protein-based primary calibrator has the advantage of behaving the closest to endogenous PCT, and no bias between calibrators and patient samples can be introduced during sample preparation (protein extraction, protein digestion). However, the actual concentration of a recombinant protein purchased from commercial sources varies greatly depending on its purity and quantitative methods (e.g., bicinchoninic acid assay, amino acid analysis). To ensure the result's accuracy and traceability to the SI units, the purity assessment and characterization of the primary calibrator are crucial. Still, it is more challenging for proteins than for peptides due to the size, the number, and the complexity of the process-related impurities (mainly proteins) generated during the production of the protein of interest [5]. Indeed, the current state of the art

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recommends using a primary reference method correcting the contribution of structure-related impurities (e.g., PICAA, corrected qNMR) [6]. To make this as straightforward as possible, it is therefore very beneficial to optimize the protein purification protocol (e.g., by combining size exclusion, ion exchange, reverse-phase chromatography) to reduce the number of impurities to be quantified. To overcome the limitations of our published method, we recently developed an absolute quantification method by ID-LC-MS/MS that relies on a protein-based calibration with an SI-traceable, well-characterized, highly purified recombinant protein.

Although it could be expected that immunoaffinity would allow improving the method's sensitivity, both methods have almost the same limit of quantification (LOQ) (0.25 µg/L for our antibody-free method and 0.5 µg/L for Tölke's method). Both are based on detecting a single tryptic peptide (LLLAALVQDYVQMK in Tölke's method, SALESSPADPATLSEDEAR in our method). Bearing in mind that these IDMS methods were developed to become secondary reference measurement procedures, a LOQ of 0.25 µg/L is sufficient to assign target values to quality control materials that could be used to monitor accuracy and comparability of routine assays but not to assign target values to calibration materials because most immunoassays have a lower LOQ.

Given that the two methods rely on two different sample preparation approaches, it will be of great interest to organize a round-robin study to compare the results provided by the two methods. This will pave the road to establish a network of calibration laboratories operating reference methods for PCT, as planned within IFCC WG-PCT [7].

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Declarations

Conflict of interest The authors declare no competing interests.

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