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COMMENTARY

Mouse tissue factor enzyme-linked immunosorbent assays: a sensitive issue

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One of my first and most important lessons as a graduate student came from the late great Michael Nesheim one quiet evening as he enjoyed a cigarette at the fume hood [1]. I had just purchased an antibody from a major biotech company and explained how I was gearing up for some groundbreaking experiments, tongue in cheek. He looked me in the eye and asked, "Are you sure the antibody works?" smoke gently wafting from his nostrils. I thought it an odd question from such a scholarly individual. "Why, of course it does. I just bought it from this reputable company, and others had published with it," was my response. In his mild-mannered way, after a good drag on his cigarette, he advised that I best test it first by immunoassay to at least have some inkling of whether it works as expected. Further, he said to make this routine practice whenever using a commercial reagent, be it a recombinant protein or antibody. Despite thinking it a waste of time and money, I did what Michael suggested and sacrificed a few microliters to appease him and the niggling doubt that had crept into my head. Lo and behold, the antibody was a dud under the conditions tested.

In this issue, Sachetto and Mackman [2] evaluated the ability of 4 commercial enzyme-linked immunosorbent assays (ELISAs) to quantify recombinant and native mouse tissue factor (TF) in various (non)physiological samples. TF is the receptor and cofactor for native and activated factor (F)VII/FVIIa, which plays a vital role in hemostasis and thrombosis [3]. Expression is upregulated in endothelial, epithelial, and smooth muscle cells, monocytes/macrophages, and extracellular vesicles derived from these cells in response to vascular injury and inflammation. Measurement of TF in plasma is notoriously difficult due to vanishingly low levels and blocking effects of FVII/FVIIa and other multivalent molecules present in plasma. In addition, the quality of mouse TF-detecting antibodies tends to be inferior to those of human TF. Activity-based assays are more sensitive than antigen-based assays for measuring TF levels but more cumbersome. Rapid and reliable ELISAs of TF are therefore highly sought-after. Findings from this study unequivocally demonstrate that the Abcam SimpleStep and DuoSet R&D Systems mouse TF ELISAs can detect picogram per milliliter concentrations of recombinant mouse TF–spiked buffer and mouse plasma, as well as TF in lysates from high TF-expressing KPC2 mouse pancreatic cancer cell line [2]. However, neither ELISA could detect native TF expression in plasma and extracellular vesicles from bacterial lipopolysaccharide-treated mice [2]. In contrast, neither the sandwich nor the competitive mouse TF ELISAs from MyBioSource detected TF in any of the samples tested. TF expression was confirmed by using an in-house TF activity assay [2]. Authors speculate that discrepancies with previously published studies using the same commercial ELISAs may be partially due to differences in sample preparation. The take-home message is that nothing trumps TF activity assays for measuring mouse TF in pathophysiological samples. The need for more sensitive, versatile mouse TF ELISAs therefore remains.

Thus, in this day and age of big team, big data science, turbocharged technologies, machine learning, and the like, it is highly advisable to take a step back and invest a little bit of time and money to test drive your reagents and assays. Know what you are using and let the community know as well. Perhaps not the sexiest science, but invaluable in the long run. As the old adage goes, junk in, junk out, and you will sleep better. Like the little Dutch kid that stuck his finger in the dyke and saved the village from imminent flooding, do your bit and save the research community from the ravages of artifacts. Research and Practice in Thrombosis and Haemostasis is very receptive to these sorts of studies and encourages you to send them our way rather than burying them in databases or theses. Research and Practice in Thrombosis and Haemostasis has a section dedicated to methodological articles in keeping

with the foundational spirit of the International Society on Thrombosis and Haemostasis to standardize protocols and reagents, having a trickle-down effect from researchers to clinicians and eventually patients. Something to bear in mind when embarking on seemingly mundane experiments this entails. Not a wasted effort in the least. Besides the invaluable lesson in the scientific process, a graduate student gains a better understanding of their reagents and fundamental techniques, possibly coming away with a concise peer-reviewed article and polished section for their thesis, whilst saving loads of other folks from the pitfalls of shoddy reagents and science.

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AUTHOR CONTRIBUTIONS

Y.A.S. wrote and edited the article.

RELATIONSHIP DISCLOSURE

Y.A.S. is an Inserm Director of Research; also served as Associate Editor for RPTH.

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