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METHODOLOGICAL ARTICLE

Evaluation of the ability of commercial enzyme-linked immunosorbent assays to measure mouse tissue factor

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Abstract

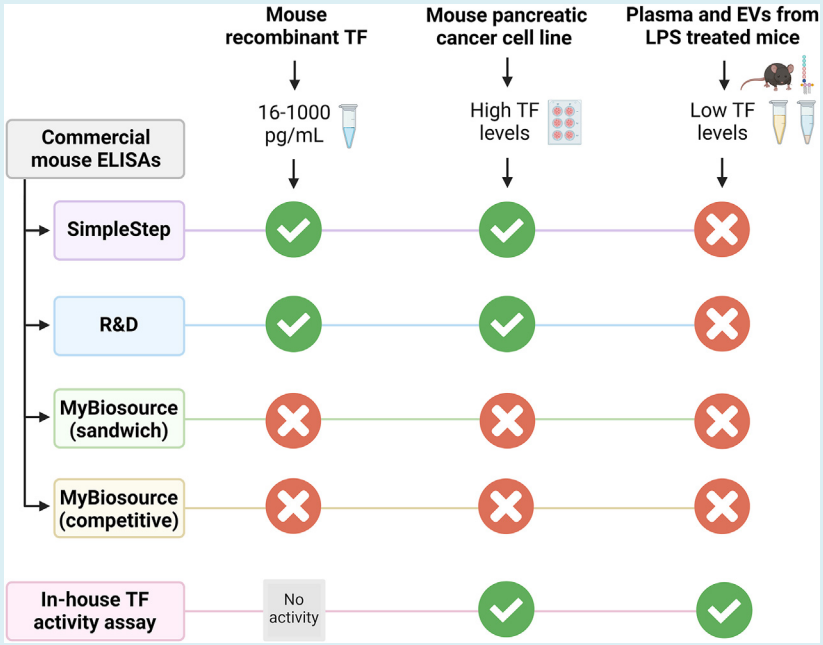
Background: Tissue factor (TF) is the primary cellular initiator of the blood coagulation cascade. Increased levels of TF expression on circulating monocytes or on extracellular vesicles (EVs) are associated with thrombosis in a variety of diseases, including sepsis and COVID-19.

Objectives: Here, we aimed to evaluate the ability of 4 commercial TF enzyme-linked immunosorbent assays (ELISAs) to measure mouse TF in cells and plasma.

Methods: We used 4 commercial mouse TF ELISAs (SimpleStep, R&D Systems, MyBioSource [sandwich], and MyBioSource [competitive]). We used recombinant mouse TF (rmTF; 16-1000 pg/mL), cell lysates from a TF-expressing mouse pancreatic cancer cell line, and plasma and EVs isolated from plasma from mice injected with vehicle or bacterial lipopolysaccharide (LPS).

Results: The 2 MyBioSource kits failed to detect rmTF or TF in cell lysates. The SimpleStep and R&D kits detected rmTF in buffer or spiked into plasma in a concentration-dependent manner. These kits also detected TF in cell lysates from a mouse pancreatic cancer cell line. A higher signal was observed with the SimpleStep kit compared to the R&D kit. However, the SimpleStep and R&D kits failed to detect TF in plasma or EVs from LPS-treated mice.

Conclusion: Our results indicate that some commercial ELISAs can be used to measure mouse TF levels in cell lysates but they cannot detect TF in plasma or EVs from endotoxemic mice.



KEYWORDS
blood coagulation, enzyme-linked immunosorbent assay, extracellular vesicles, lipopolysaccharide, plasma, tissue factor

Essentials

- Detection of tissue factor in plasma is difficult due to low levels and complexity of plasma.
- We measured mouse tissue factor antigen using 4 commercial mouse TF ELISAs.
- Two commercial ELISAs detected recombinant tissue factor and tissue factor in cells.
- None of the ELISAs detected tissue factor in plasma from LPS-treated mice.

1 | INTRODUCTION

Tissue factor (TF) is the receptor and cofactor for factor (F) VII/VIIa [1]. It is essential for hemostasis, but aberrant expression can lead to thrombosis. Mouse models have been used to study the roles of TF in hemostasis and thrombosis. For instance, epithelial cell TF is required for hemostasis in the lung and is upregulated after influenza A virus infection [2]. Administration of bacterial lipopolysaccharide (LPS) or heme to mice leads to TF-dependent activation of coagulation [3,4]. In addition, we showed that levels of TF-positive extracellular vesicles (EVs) are increased in the circulation of endotoxemic mice [5].

Measurement of TF is challenging because only small amounts are required to activate the coagulation cascade [6]. Activity-based assays are more sensitive than antigen-based assays in measuring the levels of TF. However, antigen-based assays, such as enzyme-linked immunosorbent assays (ELISAs), are widely used because they are faster and easier than activity assays. ELISAs rely on the binding of antibodies to the target antigen in samples. Information on the specificity of the antibodies used in commercial ELISAs is not provided. Another disadvantage with ELISAs is that they do not distinguish between full length TF, alternatively spliced TF, and degraded TF [7].

There are 3 major challenges with the measurement of TF in plasma compared to other samples. First, the levels of TF in plasma are very low. Second, TF in plasma will bind FVII/VIIa that may block binding of some anti-TF antibodies. Third, plasma contains multivalent substances, such as heterophilic antibodies, that can generate nonspecific signals [8–11].

In this study, we evaluated the ability of 4 commercial ELISAs to measure recombinant mouse TF (rmTF), TF in cell lysates, and TF in plasma containing TF-positive EVs. There are many commercial mouse TF ELISAs available. We selected 2 ELISAs based on the literature and 2 others.

2 | METHODS

2.1 | Commercial mouse TF ELISAs

We used 4 commercial ELISAs to measure mouse TF: Mouse Tissue Factor SimpleStep ELISA (Abcam; Cat# ab214091, lots #GR3368992-1 and #2101029785), Mouse Coagulation Factor III/TF DuoSet ELISA (R&D Systems; Cat# DY3178-05, lot #P284808), Mouse Tissue Factor ELISA (MyBioSource; Cat# MBS2512143, lot #XZND1Q4YHL;

MyBioSource sandwich), and Mouse Tissue Factor Competitive ELISA (MyBioSource; Cat# MBS722558, lot #20210903C; MyBioSource competitive). ELISAs were performed following the manufacturer's instructions. The SimpleStep kit uses a technology that is faster and claimed to be more sensitive since the antibody–analyte sandwich complex is made in a single step. The antibody and sample are incubated in solution and the tagged antibody–antigen complexes bind to the ELISA plate containing an antitag antibody. The standard curve uses 25 to 1600 pg/mL of TF and the sensitivity is 3.7 pg/mL. The R&D kit states that it detects recombinant and natural mouse TF and that it uses an anti-mouse TF antibody for capture and a biotinylated goat anti-mouse TF antibody for detection. The standard curve uses 23.4 to 1500 pg/mL. The MyBioSource (sandwich) ELISA kit uses the sandwich ELISA method, but no information is provided on the antibodies. The MyBioSource (competitive) ELISA kit employs the competitive ELISA method using a polyclonal anti-TF antibody and a TF-HRP conjugate. The TF from the samples and TF-HRP compete for the same binding sites, so when there is more TF in the sample, there is less TF-HRP binding to the plate and reduced intensity of color.

2.2 | Recombinant mouse TF

The mouse TF protein is a 294 amino-acid protein that contains an N-terminal signal sequence, an extracellular domain, a transmembrane domain, and a cytoplasmic domain [12]. rmTF (R&D; Cat# 3178-PA, lot #NPG0322091) is a truncated protein consisting of amino acids 29 to 251 and lacks the transmembrane and cytoplasmic domains. rmTF was spiked into buffer or mouse plasma.

2.3 | Cell culture and cell lysate preparation

The mouse pancreatic cancer cell line KPC2 Cas9 expresses high levels of TF, whereas the KPC2 TF knockout (KO) cell line does not express TF (kindly provided by Dr Flick, University of North Carolina at Chapel Hill) [13]. The cells were cultured in RPMI1640 medium (Thermo Fisher Scientific; Cat# 11875093) containing 10% fetal bovine serum (Omega Scientific; Cat# FB-02) and 1% Antibiotic-Antimycotic (Thermo Fisher Scientific; Cat# 15240-062). Cells were collected, washed with 1× Dulbecco's phosphate-buffered saline (DPBS), resuspended in DPBS, subjected to 3 cycles of freeze–thaw (−20 °C and 37 °C), and centrifuged at 1000 *g* for 15 minutes at 4 °C. The supernatant of the cell lysate was added to a new tube. The protein concentrations of the samples were determined using the bicinchoninic acid assay (Thermo Fisher Scientific; Cat# 23227). Cell lysates were diluted 1:4 in the recommended sample buffer for each ELISA kit.

2.4 | Mouse endotoxemia model and plasma preparation

All animal studies were approved by the University of North Carolina at Chapel Hill Animal Care and Use Committee (protocol no. 23.032-0) and comply with the National Institute of Health guidelines. We used a mouse endotoxemia model to generate TF-positive EVs as described previously [3,14]. Male C57BL6/J (8-12 weeks) were injected with LPS (*Escherichia coli* serotype O111:B4, Sigma; Cat# L2630; 7.5 mg/kg intraperitoneally) or with vehicle (DPBS). After 3 hours, whole blood was collected from the inferior vena cava into sodium citrate (final concentration: 0.38%). Blood was centrifuged at 4500 *g* for 15 minutes at room temperature and plasma was obtained and stored at −80 °C until further analysis. Plasma was diluted 1:2 in sample diluent and analyzed using ELISAs.

2.5 | Isolation of EVs and preparation of EV-depleted plasma

For the EV TF activity assay, EVs were isolated by centrifugation of 100 μL of plasma at 20,000 *g* for 30 minutes at 4 °C [5,7]. The EV pellet was washed in 1 mL of HEPES buffer saline with bovine serum albumin (HBSA, 137 mmol/L NaCl, 5.38 mmol/L KCl, 5.55 mmol/L glucose, 10 mmol/L HEPES, and 0.1% BSA; pH: 7.4), centrifuged once more, and resuspended in 100 μL of HBSA.

For ELISAs, 100 μL of sample diluent (for each ELISA kit) was added to 100 μL of plasma and the EVs were isolated by centrifugation at 20,000 *g* for 30 minutes at 4 °C. The EV pellet was washed in 1 mL of DPBS, centrifuged once more, and resuspended in 200 μL of the sample diluent for each ELISA kit. EV-depleted plasma was prepared by centrifuging the supernatant at 100,000 *g* for 70 minutes at 4 °C.

2.6 | Western blotting

Western blotting for TF was performed as described previously [13]. Mouse recombinant TF was diluted in PBS. SDS sample buffer (1× final concentration, GenScript; Cat# M00676-250) and β-mercaptoethanol (2.5% final concentration) were added to rmTF. Samples were heated at 95 °C for 10 minutes and loaded on gels (4%-20% gradient, Mini-PROTEAN TGX gels, BioRad; Cat# 456-1093). SDS sample buffer and β-mercaptoethanol were added to the KPC2 Cas9 and KPC2 TF KO cell lysates, and 40 μg of protein were added per well. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes (MilliporeSigma, Cat# IPFL00010) and the membrane was incubated with blocking buffer containing 5% nonfat dry milk in 1× tris-buffered saline, 0.1% Tween 20 detergent (TBS-T buffer) for 1 hour at room temperature. The membrane was incubated with a rabbit anti-mouse TF antibody (Abcam; Cat# ab189483, 1:1000) in Pierce Protein-Free Blocking Buffer (Thermo Fisher Scientific, Cat# 37572) overnight at 4 °C, washed, and then incubated with a goat anti-rabbit IgG-HRP (Cell Signaling; Cat# 7074, 1:5000). Membranes were developed using chemiluminescence (SuperSignal West Pico PLUS Chemiluminescent Substrate, Thermo Fisher Scien-

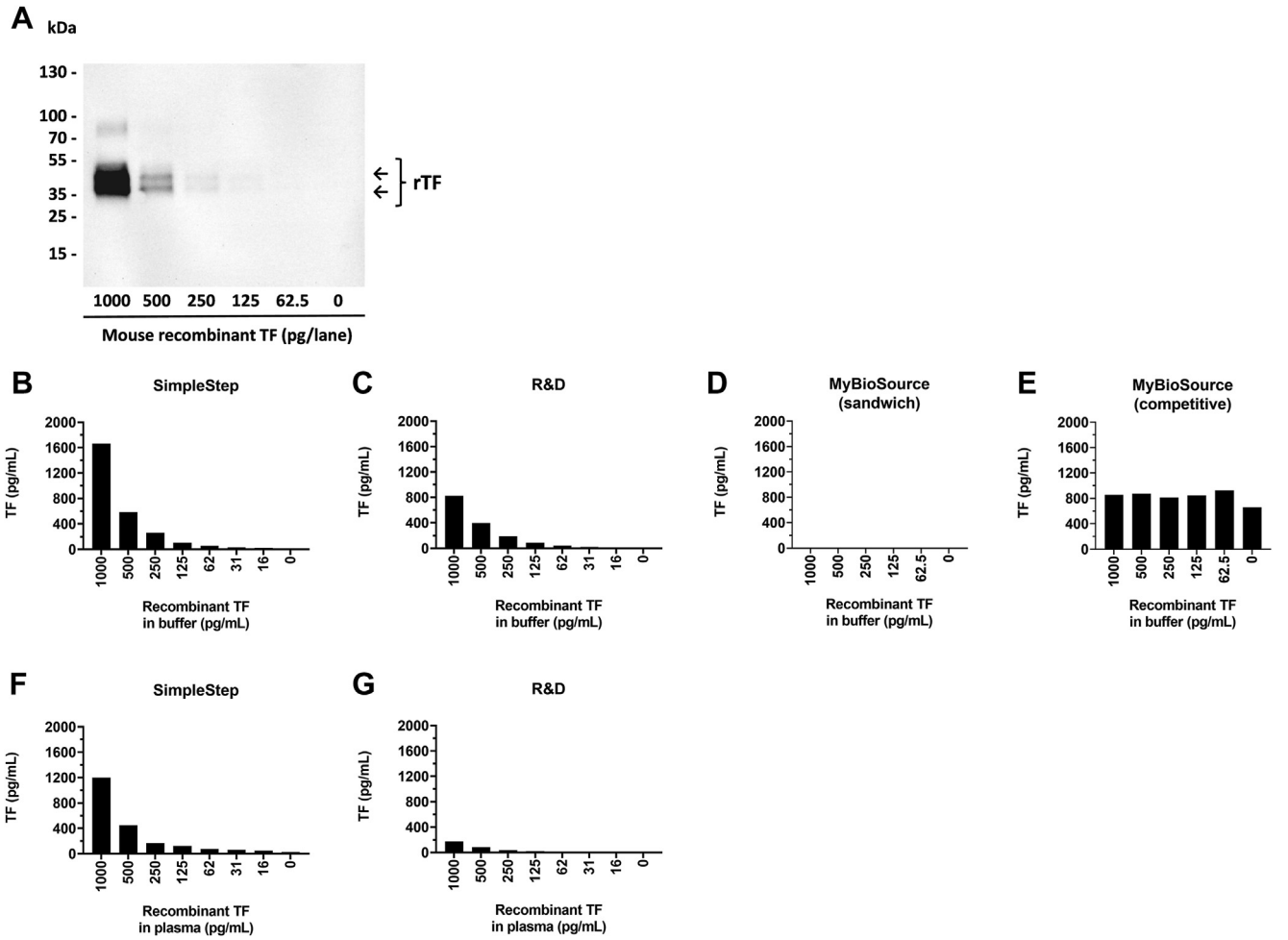


FIGURE 1 Detection of rmTF. (A) rmTF was analyzed by western blot analysis (0-1000 pg/lane). A rabbit anti-mouse TF polyclonal antibody from Abcam (#ab189483) was used (1:1000 dilution in blocking buffer; final concentration: 0.576 μg/mL). A goat anti-rabbit IgG secondary antibody (Cell Signaling, #7074) was used at a 1:5000 dilution in blocking buffer. MW standards are shown. The position of rmTF (44 and 38 kDa) is shown. Different concentrations of rmTF (either 16-1000 pg/mL or 62.5-1000 pg/mL) in buffer were measured using the (B) SimpleStep ELISA, (C) R&D ELISA, (D) MyBioSource (sandwich) ELISA, and (E) MyBioSource (competitive) ELISA. We also measured different concentrations of rmTF (16-1000 pg/mL) in mouse plasma using the (F) SimpleStep ELISA and (G) R&D ELISA. ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin; MW, molecular weight; rmTF, recombinant mouse tissue factor; TF, tissue factor.

tific; Cat# 34577) and detected using a ChemiDoc MP Imaging System (BioRad).

2.7 | TF activity assay

TF activity was measured using an in-house FXa generation assay [5,7]. Briefly, KPC2 Cas9 and KPC2 TF KO cell lysates or EVs were incubated with a rat anti-mouse TF monoclonal inhibitory (1H1, Genentech Inc [15]) or control IgG antibody to distinguish between TF-dependent and TF-independent FXa generation. After incubation with 10 nM of mouse FVIIa (a gift from Dr Paris Margaritis) and 300 nM of human FX (Enzyme Research Laboratories) for 2 hours, FXa generation was measured using a FXa chromogenic substrate Pefachrome FXa 8595 (Enzyme Research Laboratories, DSM; Cat# 085-27) for 15 minutes. A standard curve was generated using relipidated recombinant human TF (Dade Innovin, Siemens).

2.8 | Data presentation

Data are shown as individual values or mean ± SD. Normal distribution and homoscedasticity of the results were analyzed. Unpaired student t-test was used to compare groups and data were considered statistically significant when $P < .05$. Data were analyzed with Prism version 9.4 (GraphPad Software).

3 | RESULTS

3.1 | Detection of recombinant mouse TF

We analyzed rmTF by western blotting. As expected from the datasheet, we observed 2 bands at 44 and 38 kDa for rmTF that appear to be due to differential glycosylation (Figure 1A). Next, we determined the ability of the different TF ELISAs to detect different

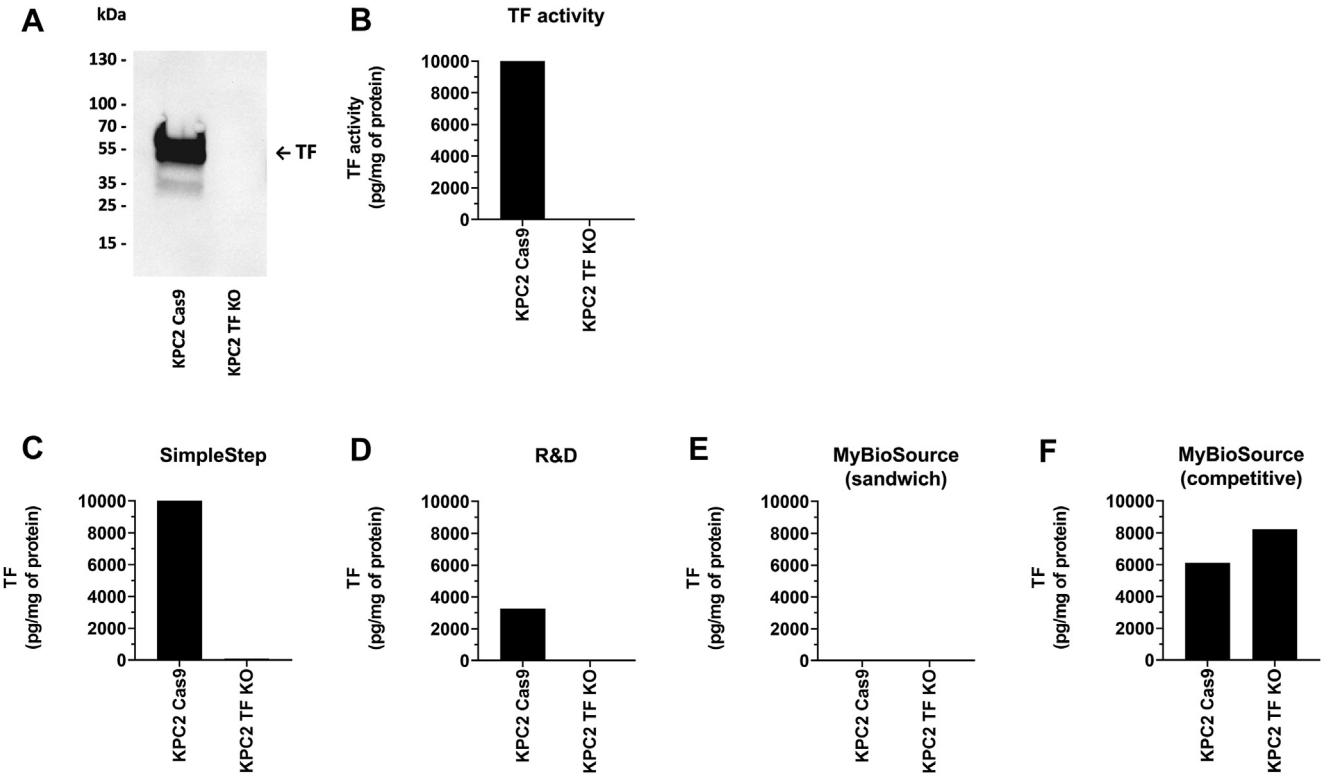


FIGURE 2 Detection of mouse tissue factor in cell lysates. We used cell lysates of a TF-positive pancreatic cancer cell line (KPC2 Cas9) or a TF KO (KPC2 TF KO). (A) Mouse TF was analyzed by western blot analysis. A rabbit anti-mouse TF polyclonal antibody from Abcam (#ab189483) was used (1:1000 dilution in blocking buffer; final concentration: 0.576 μg/mL). A goat anti-rabbit IgG secondary antibody (Cell Signaling, #7074) was used at a 1:5000 dilution in blocking buffer. MW standards are shown. The position of TF (glycosylated, ~50 kDa) is shown. (B) Mouse TF activity was determined using a factor Xa generation assay. Levels of mouse TF antigen were determined using the (C) SimpleStep ELISA, (D) R&D ELISA, (E) MyBioSource (sandwich) ELISA, and (F) MyBioSource (competitive) ELISA. ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin; KO, knockout; MW, molecular weight; TF, tissue factor.

concentrations (16-1000 pg/mL) of rmTF. Two of the ELISAs (SimpleStep and R&D) detected rmTF in buffer in a concentration-dependent manner (Figure 1B, C). Both ELISAs detected 16 pg/mL of rmTF. The signal with the SimpleStep ELISA was ~2-fold higher than that with the R&D ELISA. The MyBioSource (sandwich) ELISA did not give a signal for any of the concentrations of rmTF (Figure 1D). The MyBioSource (competitive) ELISA had a high background and did not detect recombinant mouse TF (Figure 1E). We continued studies with only the SimpleStep and R&D ELISAs. Both the SimpleStep and R&D ELISAs detected rmTF spiked into mouse plasma in a concentration-dependent manner, but the signal was lower than that observed with buffer (Figure 1F, G).

3.2 | Detection of mouse TF in cell lysates

We used the KPC2 Cas9 cell line as a positive control and the KPC2 TF KO cell line as a negative control for TF expression, as described previously [13]. As expected, we observed a band of ~50 kDa in the KPC2 Cas9 cell lysate but not in the KPC2 TF KO cell lysate (Figure 2A). This band corresponds to the size of glycosylated mouse TF. Next, we measured TF activity of the cell lysates and observed high levels in KPC2 Cas9 cells but no TF activity in KPC2 TFKO cells (Figure 2B). We determined the ability of the 4 ELISAs to detect TF in cell lysates. The SimpleStep and R&D ELISAs detected TF in KPC2 Cas9 cell lysates (Figure 2C, D). As observed with recombinant mouse TF, the SimpleStep ELISA gave a higher value than the R&D ELISA. The MyBioSource (sandwich) ELISA gave no signal, whereas the MyBioSource (competitive) ELISA gave a nonspecific signal (Figure 2E, F).

3.3 | Detection of mouse TF in plasma

We have previously shown that EVs isolated from the plasma of endotoxemic mice have TF activity [5]. We isolated EVs from the plasma of mice with or without LPS treatment as positive and negative controls, respectively. As expected, the TF activity of EVs isolated from the plasma from LPS-treated mice (2.63 ± 0.64, mean ± SD, n = 5) was higher than that of EVs isolated from the plasma of controls (0.54 ± 0.30, mean ± SD, n = 4; Figures 3A, B). In contrast to this result, we observed no difference in the signal between plasma from LPS-treated mice and controls with either the SimpleStep or R&D ELISAs (Figure 3C, F). The SimpleStep ELISA had a higher background than the R&D ELISA.

Next, we measured levels of mouse TF in EV-depleted plasma and isolated EVs.

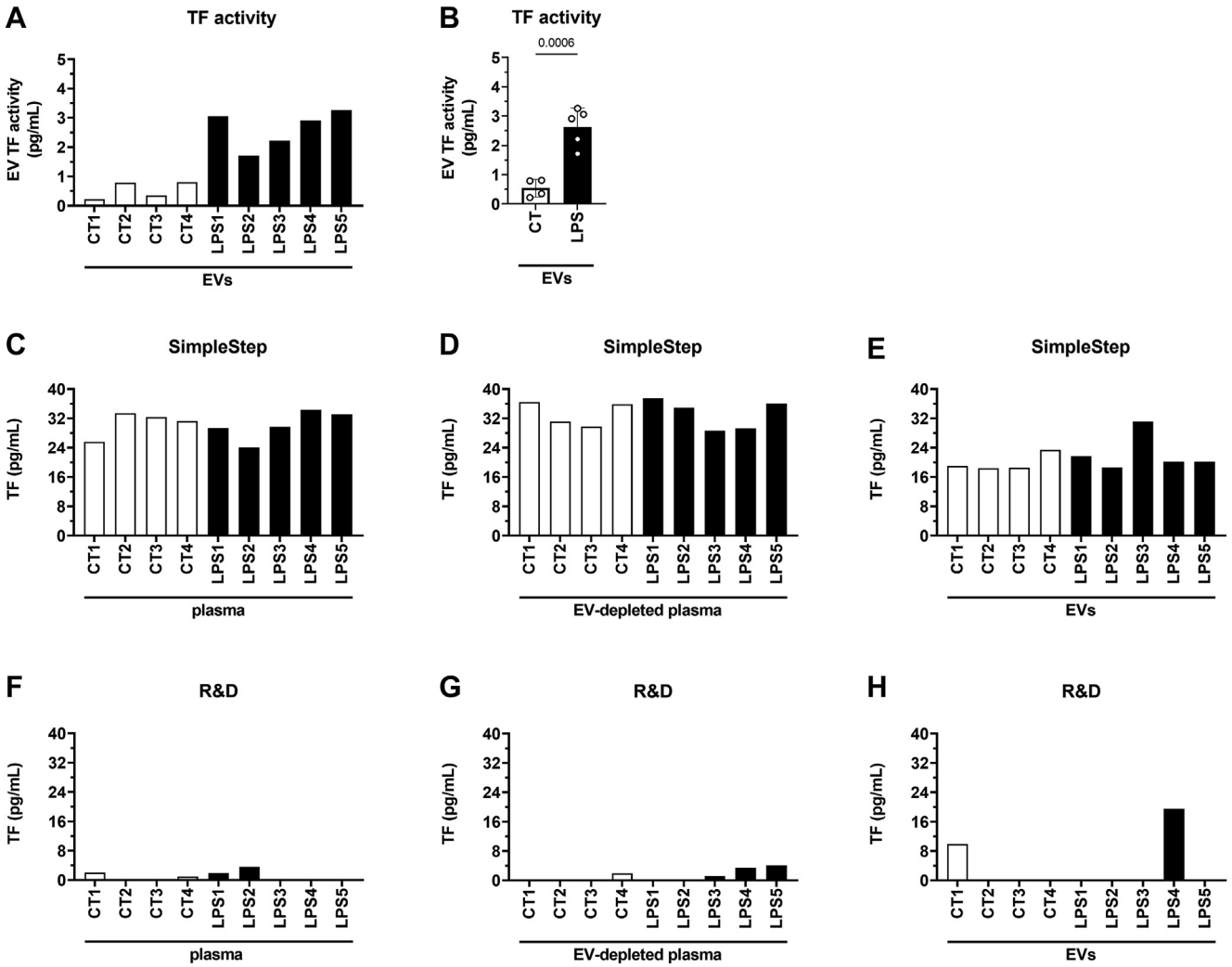


FIGURE 3 Detection of mouse tissue factor in plasma and EVs. Whole blood was obtained from 4 control mice and 6 mice injected with bacterial LPS and plasma was prepared. One LPS sample was removed after analysis using an outlier test (ROUT test). EV-depleted plasma was generated by centrifuging the supernatant at 100,000 *g* for 70 minutes at 4 °C. EVs were isolated from plasma by centrifuging 100 μL of plasma at 20,000 *g* for 30 minutes at 4 °C. The EV pellet was resuspended in 100 μL of buffer. Data are shown as individual values or mean ± SD (*n* = 4-5/group). (A, B) Mouse EV TF activity was determined using a factor Xa generation assay. Student T-test was used to analyze differences between control and LPS groups. Mouse TF antigen in (C, F) plasma, (D, G) EV-depleted plasma, and (E, H) EVs was determined using the SimpleStep and R&D ELISAs. EV, extracellular vesicle; LPS, lipopolysaccharide; TF, tissue factor.

Depletion of EVs from plasma did not reduce the high signal observed with the SimpeStep ELISA (Figure 3D). Similarly, depletion of EVs from plasma did not change the signal observed with the R&D ELISA (Figure 3G). Unlike the EV TF activity, we did not observe a difference in the signal for EVs isolated from LPS-treated mice compared to EVs isolated from control mice for both the SimpleStep and the R&D ELISAs (Figure 3E, H). Surprisingly, we observed a high signal for isolated EVs with the SimpleStep ELISA (Figure 3E).

4 | DISCUSSION

In general, commercial ELISAs that are designed to detect mouse proteins are of lower quality than commercial ELISAs designed to detect human proteins. This is, in part, due to the quality of the antibodies used. We found that only 2 of the 4 commercial mouse TF ELISAs detected both rmTF and mouse TF in cell lysates. Both ELISAs from MyBioSource failed to detect mouse TF. We observed a ~2- to 3-fold higher signal for both rmTF and cell lysates for the SimpleStep ELISA than that for the R&D ELISA, which likely reflects the use of different standards [16–18]. It is notable that the 2 MyBioSource ELISAs but not the SimpleStep and R&D ELISAs state that plasma can be used. The SimpleStep ELISA states that serum and plasma samples have not been tested (Protocol Booklet) and R&D states that DuoSet ELISAs are developed and validated with cell culture supernatants spiked with recombinant mouse TF, and additional optimization by investigators is necessary to validate the ELISAs for use of serum or plasma (https://www.rndsystems.com/resources/faqs/elisas). Interestingly, we also found that only 2 of 4 commercial human TF ELISAs detected recombinant human TF [16].

TABLE Studies measuring TF antigen in mouse plasma using commercial ELISAs

Kit	Group 1 TF antigen (pg/mL)	Disease model	Year (reference)
SimpleStep	0[a] (control) 60-110[a] (CLP)	CLP-induced sepsis	1997 [19]
SimpleStep	60 ± 9[a] (control) 235 ± 43[a] (CLP)	CLP-induced sepsis	2020 [20]
R&D	11 ± 5[a] (control) 35 ± 35[a] (*Sirt3*$^{-/-}$)		2018 [21]
R&D	37.07 ± 4.14 (control) 49.49 ± 3.17 (anti–IL-1β antibody–treated)	LPS injection + arterial thrombosis	2019 [22]
R&D	175 ± 10[a] (exposure to air) 520 ± 25 (exposure to smoke)	Smoking	2022 [23]
R&D	42-70[a] (*Tlr4*$^{-/-}$) 10-32[a] (*Tlr4*$^{-/-}$*Cd14*$^{-/-}$)	IFN-γ priming and LPS injection	2022 [24]
R&D	66 ± 7[a] (control) 82.24 ± 4.89 (CLP)	CLP-induced sepsis	2023 [25]
MyBioSource (#MBS162963)	1 ± 5[a] (control) 2.2 ± 2 (intestinal injury)	Intestinal injury	2020 [26]

CLP, cecal ligation puncture model; IFN, interferon; LPS, lipopolysaccharide.
[a]Values estimated from graphs.

The SimpleStep and R&D ELISAs failed to detect TF in mouse plasma from endotoxemic mice that contained TF-positive EVs. We searched the literature for papers that have used mouse TF ELISAs to measure TF in mouse plasma. We found 8 studies where 5 used the R&D ELISA, 2 used the SimpleStep ELISA, and 1 used a MyBioSource ELISA (Table). The background value we observed for plasma from control mice using the SimpleStep ELISA was 30.7 ± 3.5 (mean ± SD, n = 4). The 2 studies that used the SimpleStep ELISA to measure TF in mouse plasma reported values of ∼0 pg/mL and ∼60 ± 9 pg/mL (Table) [19,20]. The background value we observed for plasma from control mice using the R&D ELISA was 0.7 ± 1.0 (mean ± SD, n = 4). This is much lower than the values reported in 5 papers (range: 11-175 pg/mL; Table) [21–25]. It is unclear why there are such variations in the values using plasma from control mice, but this may be due, in part, to differences in blood collection and plasma preparation.

It is reported that levels of TF increase in the plasma of mice exposed to either cecal ligation and puncture or waterpipe smoke [19,20,23,25]. Based on our finding that the SimpleStep and R&D ELISAs fail to detect TF in plasma from endotoxemic mice, we believe that additional methods, such as measuring EV TF activity, should be employed to confirm an increase in levels of mouse TF in plasma in these models. One limitation of our study is that although endotoxemic mice have elevated levels of TF + EVs, the level is not very high and other conditions may lead to higher levels of plasma TF that can be detected by the ELISAs.

We have shown that the majority of TF in plasma from endotoxemic mice and mice bearing human pancreatic tumors is present on EVs [5,7]. Therefore, we determined if the ELISAs could detect TF on EVs isolated from the plasma of endotoxemic mice. Unfortunately, the SimpleStep and R&D ELISAs failed to detect TF on isolated EVs even though they had 1.72 to 3.26 pg/mL of TF activity.

In conclusion, the 2 MyBioSource ELISAs failed to detect rmTF and TF in cell lysates, whereas the SimpleStep and R&D ELISAs detected TF in cell lysates but not in plasma. We believe that in most mouse models levels of TF in plasma are too low to be detected by ELISA, and recommend measuring TF activity of EVs isolated from plasma.

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AUTHOR CONTRIBUTIONS

A.T.A.S. and N.M. designed experiments, interpreted data, and edited the manuscript. A.T.A.S. conducted experiments, analyzed data, and wrote the manuscript. All the authors read and approved the final manuscript.

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RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

REFERENCES

[1] Grover SP, Mackman N. Tissue factor: an essential mediator of hemostasis and trigger of thrombosis. *Arterioscler Thromb Vasc Biol*. 2018;38:709–25.

[2] Antoniak S, Tatsumi K, Hisada Y, Milner JJ, Neidich SD, Shaver CM, et al. Tissue factor deficiency increases alveolar hemorrhage and death in influenza A virus-infected mice. *J Thromb Haemost*. 2016;14:1238–48.

[3] Pawlinski R, Wang JG, Owens AP 3rd, Williams J, Antoniak S, Tencati M, et al. Hematopoietic and nonhematopoietic cell tissue factor activates the coagulation cascade in endotoxemic mice. *Blood*. 2010;116:806–14.

[4] Sparkenbaugh EM, Chantrathammachart P, Wang S, Jonas W, Kirchhofer D, Gailani D, et al. Excess of heme induces tissue factor-dependent activation of coagulation in mice. *Haematologica*. 2015;100:308–14.

[5] Wang JG, Manly D, Kirchhofer D, Pawlinski R, Mackman N. Levels of microparticle tissue factor activity correlate with coagulation activation in endotoxemic mice. *J Thromb Haemost*. 2009;7:1092–8.

[6] Mackman N, Sachetto ATA, Hisada Y. Measurement of tissue factor-positive extracellular vesicles in plasma: strengths and weaknesses of current methods. *Curr Opin Hematol*. 2022;29:266–74.

[7] Wang JG, Geddings JE, Aleman MM, Cardenas JC, Chantrathammachart P, Williams JC, et al. Tumor-derived tissue factor activates coagulation and enhances thrombosis in a mouse xenograft model of human pancreatic cancer. *Blood*. 2012;119:5543–52.

[8] Levinson SS, Miller JJ. Towards a better understanding of heterophile (and the like) antibody interference with modern immunoassays. *Clin Chim Acta*. 2002;325:1–15.

[9] Tate J, Ward G. Interferences in immunoassay. *Clin Biochem Rev*. 2004;25:105–20.

[10] Ismail AAA, Walker PL, Barth JH, Lewandowski KC, Jones R, Burr WA. Wrong biochemistry results: two case reports and observational study in 5310 patients on potentially misleading thyroid-stimulating hormone and gonadotropin immunoassay results. *Clin Chem*. 2002;48:2023–9.

[11] Bartels EM, Wätjen IF, Andersen EL, Danneskiold-Samsøe B, Bliddal H, Ribel-Madsen S. Rheumatoid factor and its interference with cytokine measurements: problems and solutions. *Arthritis*. 2011;2011:741071.

[12] Hartzell S, Ryder K, Lanahan A, Lau LF, Nathans D. A growth factor-responsive gene of murine BALB/c 3T3 cells encodes a protein homologous to human tissue factor. *Mol Cell Biol*. 1989;9:2567–73.

[13] Rosell A, Moser B, Hisada Y, Chinthapatla R, Lian G, Yang Y, et al. Evaluation of different commercial antibodies for their ability to detect human and mouse tissue factor by western blotting. *Res Pract Thromb Haemost*. 2020;4:1013–23.

[14] Pawlinski R, Pedersen B, Schabbauer G, Tencati M, Holscher T, Boisvert W, et al. Role of tissue factor and protease-activated receptors in a mouse model of endotoxemia. *Blood*. 2004;103:1342–7.

[15] Kirchhofer D, Moran P, Bullens S, Peale F, Bunting S. A monoclonal antibody that inhibits mouse tissue factor function. *J Thromb Haemost*. 2005;3:1098–9.

[16] Sachetto ATA, Archibald SJ, Bhatia R, Monroe D, Hisada Y, Mackman N. Evaluation of four commercial ELISAs to measure tissue factor in human plasma. *Res Pract Thromb Haemost*. 2023;7:100133.

[17] Parhami-Seren B, Butenas S, Krudysz-Amblo J, Mann KG. Immunologic quantitation of tissue factors. *J Thromb Haemost*. 2006;4:1747–55.

[18] Lee RD, Barcel DA, Williams JC, Wang JG, Boles JC, Manly DA, et al. Pre-analytical and analytical variables affecting the measurement of plasma-derived microparticle tissue factor activity. *Thromb Res*. 2012;129:80–5.

[19] Shimura M, Wada H, Wakita Y, Nakase T, Hiyoyama K, Nagaya S, et al. Plasma tissue factor and tissue factor pathway inhibitor levels in patients with disseminated intravascular coagulation. *Am J Hematol*. 1997;55:169–74.

[20] Xu S, Pan X, Mao L, Pan H, Xu W, Hu Y, et al. Phospho-Tyr705 of STAT3 is a therapeutic target for sepsis through regulating inflammation and coagulation. *Cell Commun Signal*. 2020;18:104.

[21] Gaul DS, Weber J, van Tits LJ, Sluka S, Pasterk L, Reiner MF, et al. Loss of Sirt3 accelerates arterial thrombosis by increasing formation of neutrophil extracellular traps and plasma tissue factor activity. *Cardiovasc Res*. 2018;114:1178–88.

[22] Liberale L, Holy EW, Akhmedov A, Bonetti NR, Nietlispach F, Matter CM, et al. Interleukin-1β mediates arterial thrombus formation via NET-associated tissue factor. *J Clin Med*. 2019;8:2072.

[23] Hamadi N, Beegam S, Zaaba NE, Elzaki O, Ali BH, Nemmar A. Comparative study on the chronic vascular responses induced by regular versus occasional waterpipe smoke inhalation in mice. *Cell Physiol Biochem*. 2022;56:13–27.

[24] Vasudevan SO, Russo AJ, Kumari P, Vanaja SK, Rathinam VA. A TLR4-independent critical role for CD14 in intracellular LPS sensing. *Cell Rep*. 2022;39:110755.

[25] Lu Y, Li D, Huang Y, Sun Y, Zhou H, Ye F, et al. Pretreatment with Eupatilin attenuates inflammation and coagulation in sepsis by suppressing JAK2/STAT3 signaling pathway. *J Inflamm Res*. 2023;16:1027–42.

[26] Namachivayam K, Mohankumar K, Shores DR, Jain SK, Fundora J, Everett AD, et al. Targeted inhibition of thrombin attenuates murine neonatal necrotizing enterocolitis. *Proc Natl Acad Sci USA*. 2020;117:10958–69.