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Application Note

Broadening the Dynamic Detection of TNF-α with Conferma® ELISAs

Introduction

Most scientists want to detect TNF-α in both normal and diseased states using a single assay, however, it is often not possible due to the "detectability" and dynamic range of the assay. Our Conferma® TNF-α ELISA addresses this through a detailed evaluation of critical reagents and in-house assay verification, allowing scientists to detect the biomarker in more samples with fewer assays and less sample.

Demonstrating Detectability

S. Fischer gave excellent insight into the importance of demonstrating detectability rather than relying on the traditional assay characteristic of sensitivity.[1] Detectability is the ability of an assay to recognize the endogenous molecule and report a value. This differs from sensitivity, which is derived from the relationship of the assay with its (usually) recombinant standard. The endogenous molecule and the recombinant standard can be very different, leading to different antibody binding characteristics and therefore sensitivity may not be equivalent to detectability.

Conferma® ELISAs are designed to provide both detectability and dynamic range as demonstrated by in-house assay verification performed with samples from "normal" and “diseased” states. **Figure 1** shows an example of this data, with all samples detected and falling well within the range of the assay.

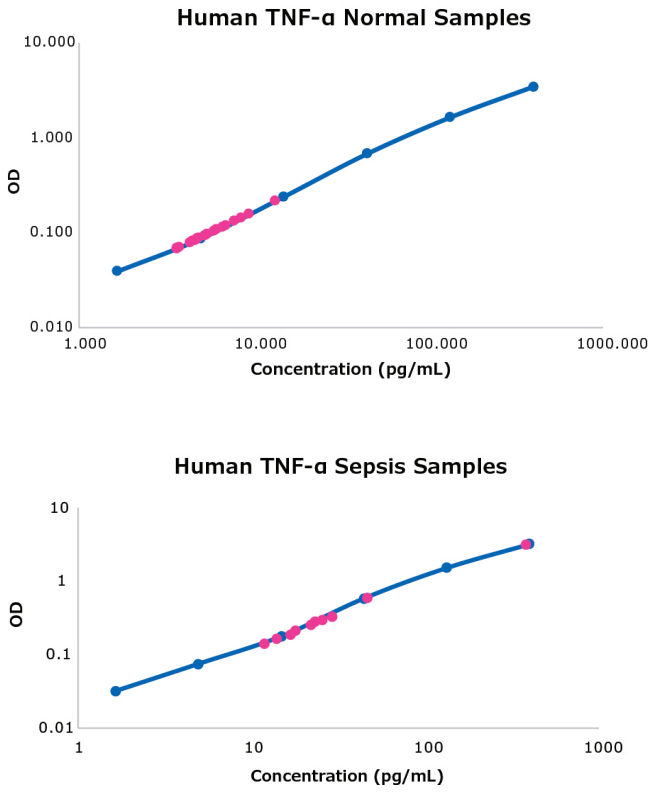

Figure 1. TNF-α concentrations were determined in normal (n = 10 serum and n = 10 plasma) and sepsis (n = 5 serum and n = 5 plasma) samples using the Conferma® TNF-α ELISA (Cat No. EZHTNFA-150K). The standard curve is represented in blue while normal and sepsis samples are represented in pink. The normal samples had a range of 3.6 – 13.2 pg/mL and the sepsis samples had a range of 11.9 – 381.0 pg/mL.

When compared to other market offerings the value is obvious. We ran "normal" samples (n=15) against two ELISAs that quantify TNF-α from the same vendor. One was the standard assay (standard curve range = 15.6 -1,000 pg/mL) and the other was the high sensitivity (HS) version (standard curve range = 0.2 – 10 pg/mL) (**Figure 2**). The Conferma® assay (Cat. No. EZHTNFA-150K, sample range = 1.65 – 400 pg/mL) correlated closely with the HS version (R^2 = 0.98). Unfortunately, most samples could not be detected by the regular version of the ELISA (3 of 15 samples were detected).

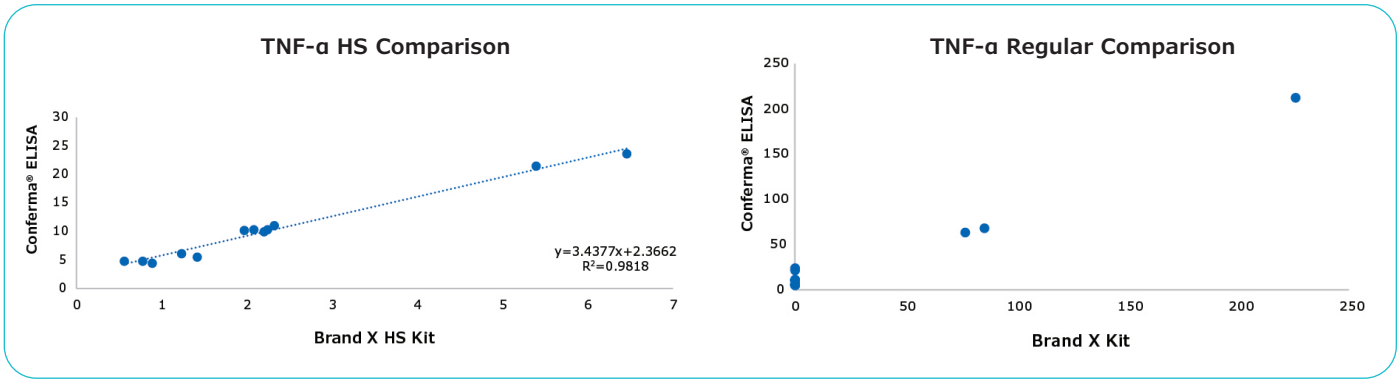

Figure 2. Detected sample correlation of TNF-α between the Conferma® TNF-α ELISA and the high sensitivity (HS) and regular versions of the competitor assay.

Between **Figure 1** and **Figure 2**, our Conferma® TNF-α ELISA demonstrates strong detectability, measuring basal levels in normal samples, allied with a dynamic range allowing diseased samples (e.g., sepsis) to also fall on the same curve. This ensures researchers will use fewer assays, less sample, and save time when using our Conferma® ELISA to detect TNF-α.

Solving the Problem with Critical Reagent Evaluation

S. Fischer concluded the 2022 Bioanalysis Zone article calling for better critical reagent evaluation, something that is key to the process behind Conferma® ELISAs.[1] Detectability is likely high when the monoclonal antibodies (mAbs) used in the assay have a strong affinity to the standard and when the standard is similar to the endogenous molecule. Conferma® ELISA reagents are evaluated lot to lot for these attributes using a suite of physiochemical characterization tools, including:

- Liquid Chromatography–Mass Spectrometry **(LC-MS)**: To examine sequence coverage
- Reverse Phase – LC-MS **(RP-LC-UV-MS)**: To compare intact mass
- Surface Plasmon Resonance **(SPR)**: To confirm antibody affinity through K_D

MSTESMIRDV ELAEEALPKK TGGPQGSRRC LFLSLFSFLI VAGATTLFCL LHFGVIGPQR EEFPRDLSLI SPLAQAVRSS SRTPSDKPVA HVVANPQAEG QLQWLNRRAN ALLANGVELR DNQLVVPSEG LYLIYSQVLF KGQGCPSTHV LLTHTISRIA VSYQTKVNLL SAIKSPCQRE TPEGAEAKPW YEPIYLGGVF QLEKGDRLSA EINRPDYLDF AESGQVYFGI IAL

Figure 3. Sequence coverage using LC-MS of the standard in the Conferma® TNF-α ELISA, TNF-α calibrator lot 44190702. The matched peptides are highlighted in cyan.

Sample	Lot No.	Mass of Dominant Peak (Da)	% Purity
TNF-α Standard H8916	ER110819	17,351	97.89
TNF-α Standard H8916	ER160519	17,351	98.37
TNF-α Standard H8916	44190702	17,351	97.57

Table 1. Analysis of the intact mass of the standard through RP-LC-UV-MS.

mAb	mAb Lot No.	Antigen	Antigen Lot No.	K_D (nM)
TNF-α c4A2 (Capture Ab1)	RB1811053	TNF-α Standard H8916	ER110819	0.28
TNF-α c4A2 (Capture Ab1)	RB1811053	TNF-α Standard H8916	ER160519	0.21
TNF-α c4A2 (Capture Ab1)	RB1811053	TNF-α Standard H8916	44190702	0.23

Table 2. The binding affinity between the standard and the capture monoclonal antibody (mAb) was determined through SPR using a Biacore™ T200 platform. Typically, a K_D (dissociation constant) value of <2 nM indicates a very high affinity between an antibody and the standard.

When physiochemical testing of Conferma® TNF-α ELISA critical reagents was performed, combining the observed sequence coverage in **Figure 3** with the intact mass analysis in **Table 1** indicated that the TNF-α recombinant standard has 80.13% similarity with the reported mature sequence of intact human TNF-α (Residues 77-233). The SPR showed that the mAbs had a high affinity to three lots of the standard as shown in **Table 2** with a K_D <0.3 nM for all three lots.

By combining high-affinity antibodies with a standard that is highly similar to the mature native protein, we achieve better detectability, as demonstrated through our in-house testing.

Summary

The Conferma® TNF-α ELISA, developed using highly characterized reagents, demonstrates excellent detectability aligned with a good dynamic range when using in-house samples.

Conferma® TNF-α ELISA Assay Characteristics

Standard Curve	1.65 - 400 pg/mL
Lower Limit of Quantification (LLOQ)	1.65 pg/mL
Endogenous Sample Testing Range (n = 20 normal, n = 20 disease state)	**Serum**: 3.58 - 16.06 pg/mL, Avg. 6.8 pg/mL **Plasma**: 3.45 - 6.88 pg/mL, Avg. 5.0 pg/mL
Spike Recovery % Range (Mean) (n=10 (5 serum, 5 plasma) spiked at 4.9, 14.8, 44.4 pg/mL)	**Serum**: 85 - 105% (95%) **Plasma**: 81 - 111% (97%)
Parallelism Range at Neat, 1:2, 1:4, 1:8 (Mean)	**Plasma**: 87 - 102% (94%)
Linearity Range at 1:2, 1:4, 1:8 (Mean)	**Serum**: 89 - 113% (101%) **Plasma**: 98 - 110% (103%)
Inter-Assay Mean %CV (Five samples on 3 plates by 3 operators)	2.26%
Sample Volume	50 µL of neat sample
Species	Human
Recommended Matrix	K2 EDTA Plasma, Serum

Conferma® ELISA Ordering Information

Product Description	Cat. No.
Conferma® TNF-α ELISA	**EZHTNFA-150K**

References

1. Fischer S. Feb 2022. Challenges with biomarker assay evaluation: endogenous analyte detectability vs assay sensitivity. Bioanalysis Zone. https://www.bioanalysis-zone.com/challenges-with-biomarker-assay-evaluation-endogenous-analyte-detectability-vs-assay-sensitivity_spotl_assay/

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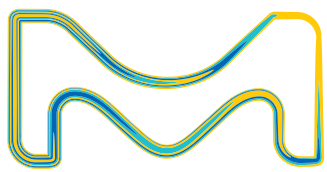

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