Development of an ELISA for the measurement of TNF-alpha in clinical samples

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Inspired by patients.
Driven by science.

Agenda

TNF-alpha as a clinical biomarker

Why develop our own assay?

Technical challenges

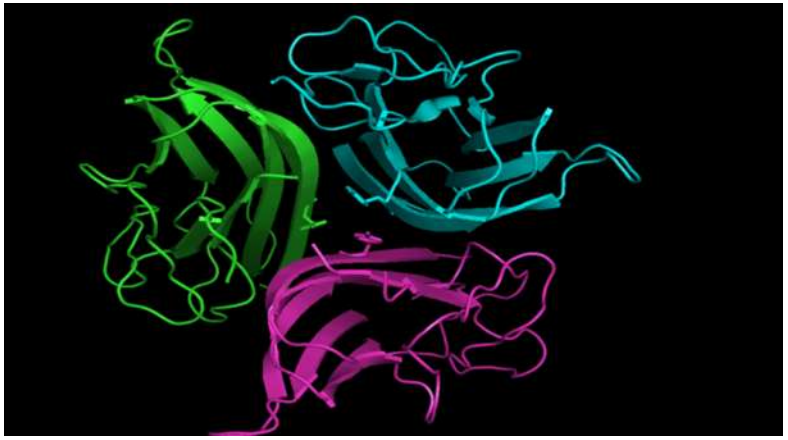
Assay characterisation and fit-for-purpose validation

High sensitivity platforms for measurement of TNF in skin homogenates

Summary and concluding remarks

TNF-alpha

Tumour necrosis factor alpha (TNF-alpha) is a homotrimeric, pro-inflammatory cytokine produced by immune cells, predominantly macrophages.



TNF-alpha is well documented to be involved in various autoimmune diseases, including rheumatoid arthritis.

An ongoing drug development programme at UCB required the accurate measurement of TNF as a biomarker for exploratory early phase clinical trials.

Commercial kits vs in-house assay development

Commercial kits

Advantages:

- Ab pairs identified, basic conditions optimised
- Wide range of options, are they fit for the intended purpose?

Disadvantages:

- Proprietary reagents
- Batch to batch variability
- Additional optimisation for a specific purpose / matrix may be required
- May not include adequate controls
- Reference material may not be well characterised and may not be representative of the endogenous analyte

In-house assay

Advantages:

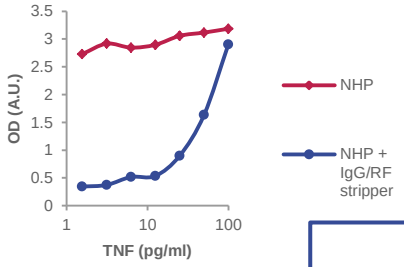
- More control over reagents (including reference material)
- Can use specialist in-house reagents
- Single batches of reagents can be made/purchased for the intended lifetime of the assay
- Can be more easily modified for programme-specific purposes

Disadvantages:

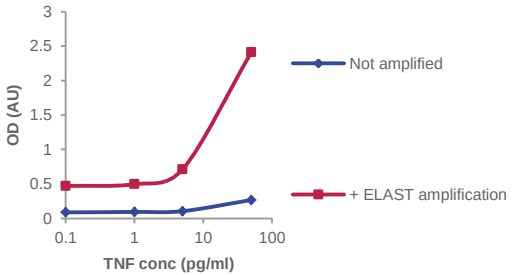
- Time consuming
- Challenges in identifying and characterising appropriate reference material

Overview of assay development

Sandwich ELISA format

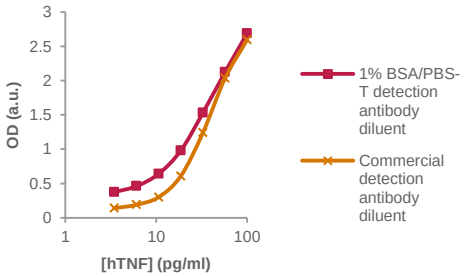


Interference in human plasma minimised using commercially available diluents



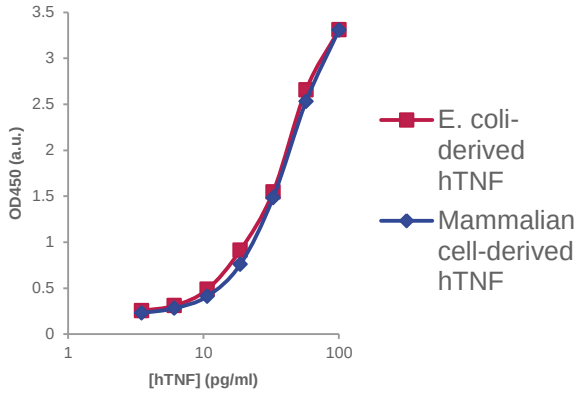
Sensitivity increased using the Perkin Elmer ELAST kit

Optimisation of detection antibody diluent to increase sensitivity



Provisional LLOQ ~ 10pg/ml

Can the assay detect native TNF?



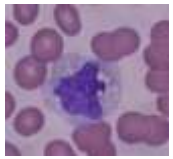
Mammalian-derived TNF can be measured using an *E. coli*-produced recombinant TNF calibrator

Native endogenous TNF produced in whole blood in response to stimulation with LPS can be detected in plasma

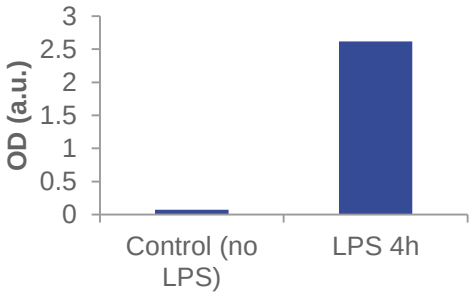
Blood sampled

in vitro stimulation of whole blood with LPS

Activation of TLR-4 on surface of monocytes and macrophages leads to increased TNF synthesis



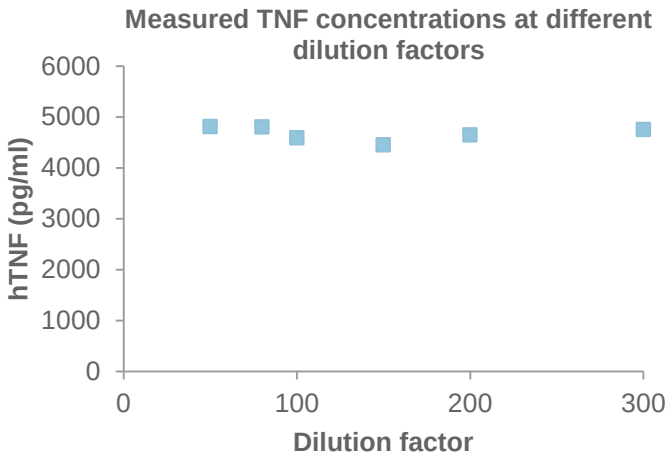
Soluble TNF-alpha in plasma increased to measurable levels



Further characterisation of the method

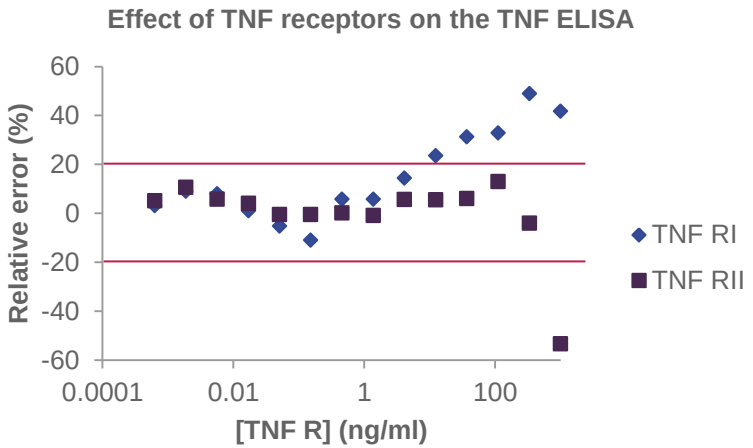
Parallelism

- Plasma from *in vitro* LPS-stimulated whole blood was tested at a range of dilutions
- High degree of precision between the corrected results from different dilutions, indicating good sample parallelism



Effect of soluble TNF receptors

- Soluble TNF receptors were titrated into a single TNF spike concentration to determine the effect on TNF measurements in the presence of endogenous binding proteins
- Soluble receptors only had an effect at high, non-physiologically relevant concentrations

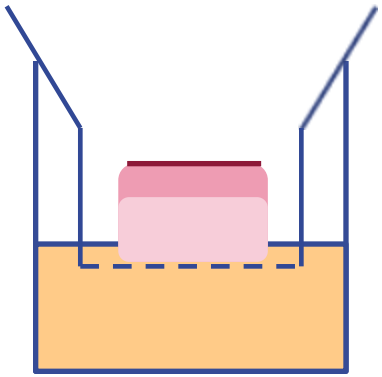


Application of method to skin homogenates

Higher degree of sensitivity required

For method development fresh healthy human skin stimulated with LPS to increase TNF levels

Singulex is being considered to increase sensitivity further



Skin incubated in transwells in serum-containing medium
+/- LPS

Homogenised and centrifuged. TNF measured in supernatant by MSD or ELISA

Summary & concluding comments

An ELISA has been developed in-house to measure TNF in plasma and skin

Technical challenges have been overcome to detect and quantitatively measure endogenous TNF in plasma samples, with good parallelism and in the presence of soluble receptors.

The method has successfully been transferred to support future clinical sample testing.

Work is ongoing to measure TNF in skin. Singulex may be used for increased sensitivity.

Key learnings include the advantages in certain situations of developing bespoke immunoassays and the challenges involved in applying these to complex matrices.

Supports the programme by delivering a means of measuring TNF as a biomarker in various matrices to support clinical studies

Acknowledgements

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Thanks!