

Cytokine Quantification in Drug Development: A comparison of sensitive immunoassay platforms

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Abstract

Immuno-PCR (IPCR) and ElectroChemiLuminescence (ECL) are technologies for improved performance of immunoassays. The specific advantages of the different technologies, using enzymatic or electrical signal amplification, allow for tailored assay design and address several specific challenges, such as maximum sensitivity up to 1 fg/ml target cytokine, simultaneous detection of multiple antigens, and high robustness against biological matrices.

Introduction

Since the development of easy and convenient ELISA as a prime tool for routine laboratory immunoanalytics, great advances have been made in assay design and assay optimization. This progress in assay technology complements the demands of modern drug development for new tools in monitoring novel compounds and the resulting alterations in biomarker profiles as triggered by application of the drugs. Particularly the profiling of cytokine patterns gives in-depth diagnostic information such as inflammatory symptoms and allows for analyzing body response to both, diseases and treatments[1]. The key factor for a meaningful analysis of biochemical interactions leading to efficient drug development based thereon, is the quality of assay technology. While the central component of any good immunoassay is the high performance of target-specific antibodies, antigen recognition is only one important issue of the assay. The other main parameter is the detection of the binding event, finally defining sensitivity and robustness of target quantification.

Here, the comparison of different properties of certain immunoassay technologies is essential for the selection of the optimal assay format for the intended application.

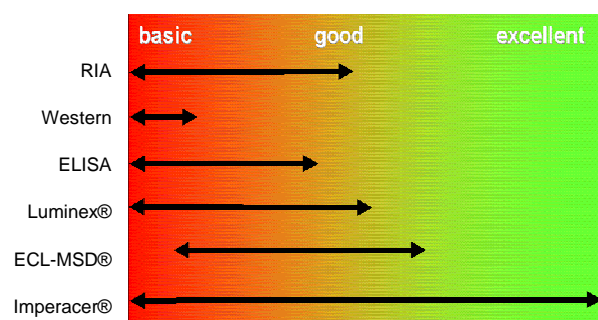


Figure 1. There are many different immunoassay formats available: Selection of the appropriate detection window for the intended application is the key for efficient analysis.

ELISA, ECL and Immuno-PCR

Today, a broad range of immunological detection techniques are available for the analytic scientist, starting with “classical” enzymatic amplification of different substrates (e.g. photometric, fluorescence and chemiluminescence-generating compounds), more sophisticated signal-enhancement technologies and, finally, ultra-sensitive assays with exponential signal amplification. The choice of the correct detection method is thereby naturally a compromise of different parameters, namely (I) the demands of the assay (*Figure 1*) and (II) the requirements of the analytic procedure in terms of reagents and instrumental equipment (*Figure 2*).

While it makes very limited sense to use a highly sensitive assay format for the qualitative detection of high target concentrations, the need for a broad detection window, extremely high sensitivity and/or reliable robustness in the presence of biological matrices present additional challenges for assay design. The adaptation of a given immunoassay protocol to these kinds of challenges is a stepwise procedure of method enhancement, after selecting the best tool for a given laboratory application. For expanding properties of conventional ELISA, mainly two analytic technologies supplement each other in regard to increased sensitivity and routine handling:

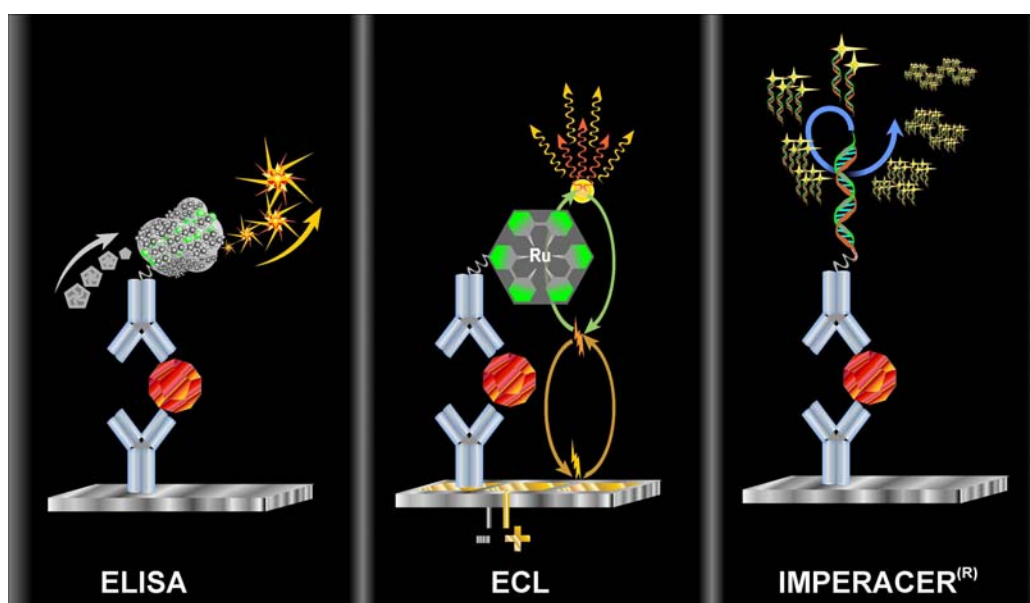


Figure 2. Sensitive assay formats and technologies for signal enhancement: Two specific antibodies (blue) recognize the analytic target (red). In classical Enzyme Linked Immuno Sorbent Assay (“**ELISA**”), a linear signal amplification is accomplished by conversion of one molecule substrate to one molecule product, each by antibody-enzyme conjugates. In Electro-ChemiLuminescence (“**ECL**”), a reaction cascade transfers electrical energy from plate surface to a light-emitting antibody / transition metal complex. The most efficient signal enhancement is accessible by exponential DNA-amplification of antibody-DNA conjugates, effectively doubling the amount of signal-generating marker in each cycle of real-time PCR read-out in **Imperacer**[®] detection. For signal generation & detection, an appropriate ELISA microplate reader, a specialized ECL instrument (**Fig 3A**) or a (real-time) PCR cycler are utilized.

With ElectroChemiLuminescence (“ECL”) [2], an increased sensitivity over a broad dynamic range is combined with a convenient multiplex-protocol. The optimized plate and instrument components of the Meso Scale Discovery (MSD[®]) assay system are working well together for providing fast and convenient, automated, high-throughput solutions (**Figure 3A**).



Figure 3A. Meso Scale Discovery (MSD[®]) ECL-detection system: an optimized combination of functional plate material and automated reader for high-throughput multiplex analysis.

A different assay design is utilized by the ELISA-analogous Imperacer[®] technology, which combines quantitative real-time PCR and ELISA to the most sensitive immunoassay format routinely available today[3, 4]. The modular assay set-up and the usage of common real-time PCR instruments for signal-read-out are making this assay concept very flexible for fast adaptation to different analytic challenges. Additionally, the benefits of sandwich target identification and extreme sensitivity enables supreme assay robustness by application of tailored AnySource[®] sample dilution strategies. Imperacer[®] is, therefore, the method of choice for the hardest of analytic challenges (**Figure 3B**).



Figure 3B. Imperacer[®] tailored kit solutions for ultra-sensitive real-time antigen target detection.

Supreme Sensitivity

When comparing immunoassay sensitivity in cytokine quantification, human Tumor Necrosis Factor alpha (TNF α) may illustrate the stepwise gain in sensitivity (**Figure 4A**). Even the most optimized standard ELISA protocol for this versatile biomarker reveals merely a quantification limit of approx. 2.000.000 molecules/well (0.5 pg/ml). Thereby, in a way sacrificing versatility for sensitivity with very narrow detection windows. In comparison, ECL shows already enhanced sensitivity to ELISA (1.1 pg/ml in human serum, no data available for the detection limit in a buffer system) and increases the quantification range to approx. 4 orders of magnitude target concentration in a single assay set-up. This advantage is caused by the specific hardware-triggered signal-generation step of the electrochemiluminescence method. However, Imperacer[®] is surpassing conventional detection limits with a detection range of approx. 0.001 pg/ml (1200 molecules/well) – 1000 pg/ml (0.01 pg/ml in human serum;

0.001 pg/ml in buffer), thereby underlining the potential of modern immunoassays when challenged for maximum performance. A brief comparison between MSD[®] and Imperacer[®] on the limits of detection (LOD) of a set of human cytokines in human serum samples is given in **Table 1**.

Cytokine	LOD Imperacer [®]	LOD MSD [®]
IL-2:	0.1 pg/ml	1.3 pg/ml
IL-6:	0.1 pg/ml	0.7 pg/ml
IL-8:	0.1 pg/ml	0.3 pg/ml
IL-11:	0.8 pg/ml	Not available
IL-12:	0.05 pg/ml	No Data
IL-17:	0.02 pg/ml	0.4 pg/ml (Tissue culture)
IL-23:	0.2 pg/ml	Not available
INF γ :	0.3 pg/ml	4.4 pg/ml
TNF α :	0.01 pg/ml	1.1 pg/ml
TNF α in buffer:	0.001 pg/ml	0.7 pg/ml (cell culture supernatant)

Table 1. Comparison of the sensitivity levels of certain cytokines in human serum. The lower limit of detection (LOD) for Imperacer[®] assays is defined as the value for the negative control plus 3-times its standard deviation. For MSD[®] the LOD is defined as the NC value plus 2.5-times its standard deviation (www.meso-scale.com).

Multiplex, PolyPlex[®] and AnySource[®]: Getting more out of one sample

Besides the race for ultimate assay sensitivity, each detection method has its own inherent advantages. ECL detection technology allows for the detection of multiple spots in each well of the special MultiArray[®] / MultiSpot[®] microplates, thereby adding additional parameter testing to a given assay format. Ready-made kits for simultaneous, multiplex detection of up to 10 cytokines are readily available for sample profiling.

In Imperacer[®], the optimized AnySource[®] approach allows for trace target detection in minuscule amounts of sample material, e.g. few μ l of biological material from small animals are sufficient for several assays. Hard-to-access sample material, such as Cerebrospinal fluid (CSF), can be substituted by easily accessible matrices, such as serum, as lowest levels of target antigen migrated into these matrices becomes detectable. Recently, it was also demonstrated that invasive sample collection techniques (e.g. drawing ml amounts of venous blood) can be substituted by less stressful procedures (e.g. sampling few hundreds of microliters of capillary blood from the earlap) or even non-invasive sampling due to sensitive low-level detection with Imperacer[®]. This allows for parallel PolyPlex[®] detection of different biomarkers e.g. in saliva samples [5].

Furthermore, special background challenges, like interfering compounds and matrix effects of biological samples, can be compensated by pre-dilution of the sample using tailored buffer systems. Imperacer[®] allows for an extreme increase in signal intensities (due to the exponential nature of the real-time PCR detection) and thereby sensitivity-increase for very small absolute amounts of antigen. This leads to the possibility of increased sample dilution ratios. The AnySource[®] sample dilution technology is specifically designed to support Imperacer[®] assays, with the combined advantages becoming obvious e.g. for analysis of serum sample material (**Figure 4B**).

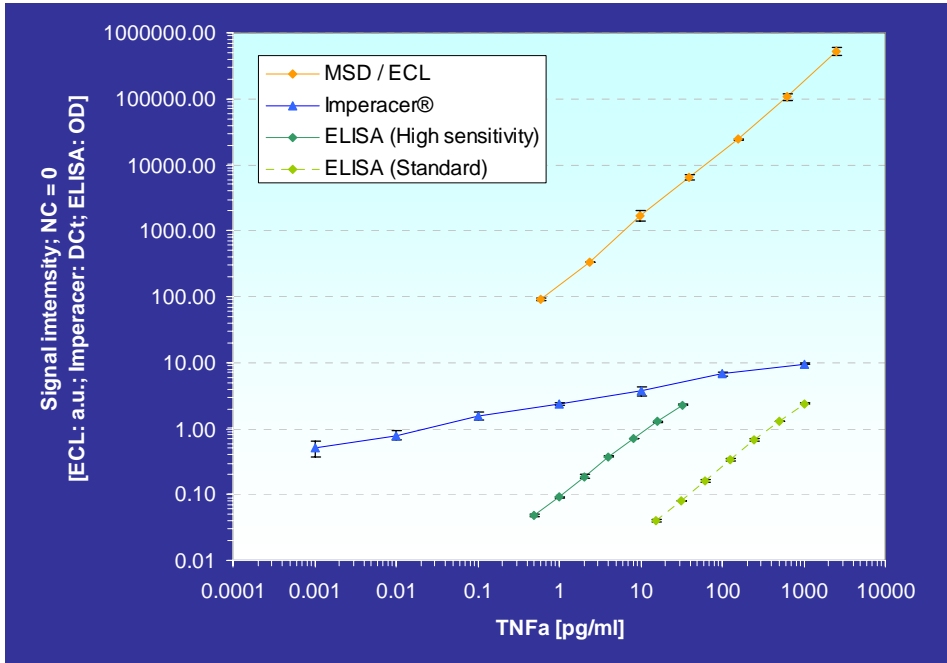


Figure 4A. Comparison of sensitivity for different analytical techniques (see also Figure 2): ELISA-assays (green) are typically covering only a narrow detection window. ECL expands the possibilities of immunoassays by an extremely improved dynamic detection range. Maximum sensitivity is accessible with Imperacer® signal amplification.

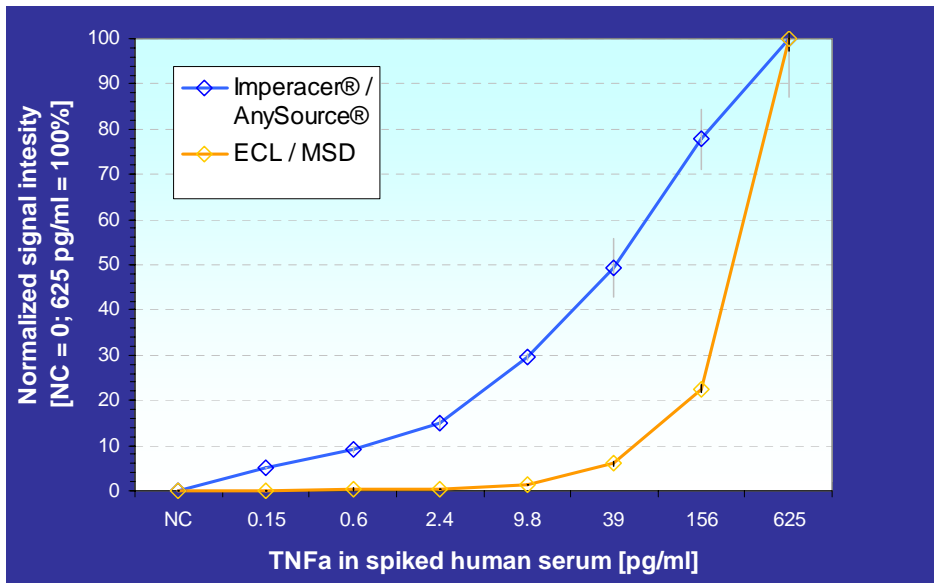


Figure 4B. Application of AnySource® signal enhancement in biological matrices: Interfering compounds from human serum are neutralized by Imperacer® / AnySource® combination, allowing for robust detection of minuscule target antigen concentrations in only 7.5 µl of required serum sample material.

Coming back to the issue of antibody quality, there are many antibody pairs which are unsuited for simultaneous multiplex analysis due to unspecific interaction with each other. Here, sensitivity also offers an alternative strategy by dilution and subsequent division of a given sample in multiple separate assays (in contrast to the multiplex approach to detect multiple marker in one well). This kind of Polyplex[®] analysis[3, 4] enables an elegant way to determine multiple analytes with highest possible sensitivity and specificity from a single source, even when only small amounts of sample material are available and antibodies or analytes are incompatible with standard multiplex protocols. Once again, the sensitivity of an Imperacer[®] assay supplements other assay formats for the most challenging of applications.

Different assay formats are thereby complementary to each other, enabling high-throughput screening of multiple compounds as well as ultra-sensitive quantifications in difficult sample materials.

Conclusions

The properties of novel immunoassay formats open up new fields of application for the analytical scientist. With the increasing understanding of genomics and proteomics, the amount and depth of analytical problems is also ever growing. When the requirements of sample material and detection level are beyond the scope of conventional ELISA, the appropriate solutions are nevertheless already available. However, it is necessary to decide which parameters are essential for the given analytical challenge.

For routine applications on a 'basic' sensitivity level, ELISA might be sufficient and generally provides the lowest costs per analysis. MSD[®] provides an excellent tool when it comes to immunoassays in the 'basic' to 'very-good' sensitivity level range and it is the method of choice for performing multiplexing studies in this sensitivity range. In cases where the 'maximum' sensitivity is needed, or where issues like low sample volumes or matrix effects need to be compensated, Imperacer[®] is the most powerful alternative.

Therefore, state of the art, well-optimized, ultra-sensitive immunoassay formats provides the tools for the challenges of tomorrow.

References

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