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Solid-phase and bead-based cytokine immunoassay: A comparison

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Abstract

Cytokines and chemoattractive cytokines (chemokines) are present in a wide variety of body fluids such as plasma, cerebrospinal fluid, bronchoaveolar fluid, amniotic fluid, synovial fluid, middle ear effusion fluid, and urine. Cytokines can be detected using classical solid-phase sandwich immunoassays such as enzyme-linked immunosorbent assay (ELISA) or with a bead based multiplex immunoassay (MIA). The physical chemical properties of the different body fluids (such as pH and total protein content) differ, which may have an impact on the outcome of the cytokine assay. Both ELISA as well as MIA cytokine detection systems are constructed by sandwiching the protein of interest between a capture and reporter antibody. When the biological sample contains heterophilic antibodies (such as in patients with auto-immune diseases), these non-specific antibodies can cause false positive results. During pathological conditions, cytokines may be found over a wide concentration range; likewise have to cover this dynamic range in a similar fashion. The correct (statistical) analysis of standard curves and (multiplexed) data are critical for proper interpretation. Classical ELISA based cytokine assays are robust, easy to use and very well suited for measurement of single cytokines. Due to an increased interest in the integral approach to understand biological processes (the omics era), multiplex immunoassays for detection of cytokines and the interpretation of these assays are gaining popularity.
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1. Introduction

Cytokines are small, non-structural glycoproteins that serve as messenger proteins between cells and are involved in such diverse processes as cell growth, cell differentiation, tissue repair and remodeling, and regulation of the immune response. Understanding cytokine function and interactions is crucial to unravel the complex cellular communication networks and therefore detection of these soluble mediators has been of interest over the last four decades. Because cytokines can serve as potential biomarkers for health and disease and changes in cytokine levels can serve as readout systems for therapeutic interventions, cytokine research has been and is the focus of many researchers. Soluble factors involved in the pathogenesis of a variety of diseases were described long before the appropriate technology to detect these soluble factors had been developed. Initially, cytokines were referred to as soluble lymphocyte factors or lymphokines. This nomenclature was based on the origin of the factor (the lymphocyte) and its function (kinesis, meaning movement, and change) [1]. Historically, the nomenclature for individual soluble factors was based on their biological activity, or the activity in a particular in vitro assay (macrophage inhibitory factor). However, the situation became more complex when it appeared that these proteins shared structural similarities as well as overlapping functions. In 1979 a consensus group

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of scientist introduced the term interleukin. Interleukins are part of the cytokine family of proteins and have a molecular weight between 4 and 50 kDa. A sub-family of the interleukins are the chemoattractive cytokines (chemokines) which have a molecular weight between 4 and 16 kDa but a completely different function. Unfortunately, this nomenclature is imperfect because the term interleukin does not always reflect the biology of the cytokine. For example, interleukin 8 is a chemokine but has an interleukin classification, whereas others such as CCL11 (Eotaxin) do not.

Secreted interleukins interact directly with surrounding cells and tissue. Cytokines are effector molecules with the ability to operate independently to regulate production as well inhibit activity of cytokines and other inflammatory mediators and thus can alter the behavior of the immune system instantly during an immune response [2,3]. The method of cellular communication by cytokines can be either autocrine, paracrine, endocrine or juxtacrine, and meaning, respectively, that the cytokines act only on the cells that secreted them, on nearby cells via diffusion, on all cells of a given tissue, or that the cytokine remains cell-bound and activates receptors on immediately neighboring cells. Chemokines on the other hand are regulatory molecules which attract and direct differentiation of new potentially inflammatory cells to a site of inflammation [4–6]. These characteristics imply that measurement of a single cytokine is often not sufficient to gain a realistic impression of the complexity of biological interactions at the cellular level. The meaning of a certain pg concentration of a given cytokine should always be considered in the context of all other cytokines present. A large number of methodologies have been developed and employed for quantification of cytokines. Immunoassays, such as solid-phase enzyme-linked immunosorbent assay (ELISA) are currently the most commonly used technique to quantify cytokines due to the high specificity and sensitivity [7]. Build on the same principle, more rapid, sensitive, automated, and high throughput methods are expanding the repertoire of techniques available to explore, diagnose, and clinically manage disease via cytokine detection. This review focuses on detection of human cytokines using sandwich immunoassays such as solid-phase ELISA and bead based multiplex immunoassays (MIA) with the Luminex platform.

Table 1
Comparison of characteristics between three techniques for cytokine detection, bioassays, solid-phase enzyme-linked immunosorbent assay (ELISA), and multiplex immunoassays (MIA)

Bioassay	ELISA	MIA
Highly sensitive: <1 pg/ml	Sensitive: <10 pg/ml	Highly sensitive: a few pg/ml
Semi-quantitative detection	Quantitative detection	Quantitative detection
Low specificity	High specificity	High specificity
Detection of bioactive molecules	Detection of bioactive and inactive molecules	Detection of bioactive and inactive molecules
Narrow analytical range (cytokines)	Wide analytical range	Growing analytical range (cytokines, phosphoproteins, antibodies, and HLA)
Time consuming (24–96 h)	Relative rapid (day)	Rapid (hours)
Low Precision (CV = 20–100%)	Excellent precision (CV = 5–10%)	Good precision (CV = 10–15%)
Drug interference	No drug interference	No drug interference
Laborious protocol	Simple protocol	Simple protocol
Large sample size	Large sample volume	Small sample volume
No simulations detection	No simulations detection	Simultaneous detection
Applications for human, mouse, and rat	Applications for all species	Applications for multiple species
High staff cost	High reagent cost	Initial set up time is consuming and expensive

2. The ideal immunoassay

As stated above, cytokines originally were defined by their ability to modify biological functions of cells. Based on these properties, bioassays have been developed for measurement of (functional) cytokine concentrations. Bioassays at best are semi-quantitative, have a low specificity, and are time consuming (Table 1). Immunoassay of cytokines offers a number of advantages over bioassays (Table 1).

Whatever methodology is developed for detection of cytokines, a number of parameters are of critical importance. When these parameters are optimal, the perfect assay would have been created. However, often one or more parameters are conflicting or suboptimal and thus it will be difficult if not impossible to create a perfect assay. (i) First, all reagents should have a high specificity and affinity as well as high sensitivity for the specific mediator for which the assay is designed. When an assay has a high sensitivity, samples sizes can be small, which is important when multiple assays have to be performed. A high sensitivity also may facilitate early diagnosis of pathological conditions by detecting lower relevant cytokine concentrations. (ii) Furthermore a simple protocol will minimize errors. Omitting all washing steps and decreasing incubation times will save time and automation can be easily introduced when a simple protocol is developed. However, simplified protocols will often reduce sensitivity and specificity. (iii) Reproducibility and reliability are critical for the use of any immunoassay in clinical studies. Internal control samples will provide information of intra and inter assay variation. Using this kind of standardization a reproducible and reliable immunoassay can be performed at different time points and at different locations and thus create a robust assay. (iv) An immunoassay should be time and cost effective. A rapid analysis will be desirable in a clinical setting and will improve high throughput screening. Budgets in a research setting are always tight and therefore cost will always be an issue. (v) Finally, it would be desirable to have the possibility for (simultaneous) detection of multiple analytes from a single small sample. This will increase data output and reduce sample sizes, but will increase cost.

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