Miltenyi Biotec

Your guide to side-by-side cytokine comparison

This document provides an overview of the benefits and processes of side-by-side testing of cytokines. It also lists some of the situations where this approach will not succeed.

Why should you consider side-by-side testing?

Efficient cellular production relies on knowing the biological activity of the cytokines employed. This ensures a high degree of reproducibility and keeps costs to a minimum. Although most suppliers of clinical-grade cytokines provide activity data for each cytokine batch, these values are not necessarily accurate (please see our application note "The reliability of stated cytokine activities" for more information).

There are two main causes for such variabilities. Note that these can even be observed for cytokine activity calibrated with an international standard.

- Significant variability can arise if different cell lines or a substantially different protocol are used to determine activity. In such cases, even calibration with an international standard can only reduce variation by a certain extent.
- The biological effects of cytokines are complex and depend on various factors, including the target cell and its environment, the overall cell culture conditions, and synergistic effects with other cytokines and cofactors. It is not possible to express all these potential effects in a single numerical value. Therefore, biological activity only gives a simplified impression of the potency of a cytokine without any guarantee that this will transfer one-to-one into a specific application.

In summary, the results of measuring cytokine activity can vary depending on the cell line and protocol. They are a highly simplified expression of a complex biological effect.

We recommend:

- Testing the cytokine in the intended application to check how well the supplier's activity values translate to that application.
- Comparing cytokines from different suppliers to find the one with the best activity in the intended application and to detect any inconsistencies in the stated performance.

What systems are best for measuring cytokine activity?

Determining cytokine activity requires both biochemical methods and cell-based bioassays[1]. The latter yields the dose-response curve (fig. 1) for the active cytokine which displays how much cytokine is necessary to induce a certain measurable biological response such as proliferation, differentiation, cytotoxicity, or cytokine secretion.

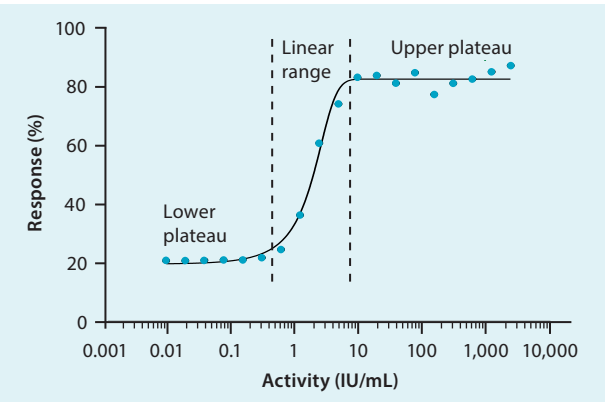

Figure 1: An example of a dose-response curve. The curve can be separated into a lower plateau (cytokine stimulus has yet to yield a measurable response), a linear range (the response is proportional to the cytokine stimulus) and an upper plateau (additional cytokine stimulus does not increase the response).

Cell-based bioassays should ideally offer good precision (i.e., repeated measurements of the same sample should yield comparable results), a broad dynamic range (the linear range should cover a wide range of response), and a good signal-to-noise ratio (the ratio of the upper plateau to the lower plateau). They provide a reliable way to quantify the cytokine activity.

Our approach to cytokine activity data
Miltenyi Biotec displays specific cytokine activities determined using the described bioassays that are calibrated to international standards[2] whenever possible. To learn more about activity determination or calibration, please refer to our application note "Recombinant cytokines and their biological activity".

Choosing your test system for side-by-side comparisons
The intended application is the best system for comparing cytokine products from different suppliers, provided the application responds proportionally to the cytokine stimulus and that an easy readout is possible.

Ideally, comparing cytokines in a scaled-down version of the intended application will not require much setup. In most cases, the researcher already has a very good understanding of their application and should have at least a rough idea of the relevant parameters for a correct interpretation of the results. This means that they know:

- The lower and upper plateau responses and approximately which cytokine concentrations are necessary to elicit these responses.
- The robustness of the assay to cytokine variations. During production, high robustness is an advantage, but when testing and comparing cytokines some differences in activity might not be visible if the assay is "too" robust. To interpret the results correctly, the researcher should know the minimum detectable cytokine difference.

How should you set up your side-by-side comparison?

A side-by-side comparison assay is essentially a parallel titration of the cytokines that you are comparing. You will need to know the approximate cytokine activity required for your application as you will use it as a starting point.

Begin by assessing whether this activity value is in the saturation phase for this assay (the upper plateau; fig. 2A). If so, the titration will only go down (i.e., to lower cytokine activity) until you reach the linear phase of the assay. That will allow you to compare the cytokines. If the starting cytokine activity is in the linear range of the assay (fig. 2B), the titration will go up and down (i.e., to higher and lower cytokine activity).

When choosing the titration steps, ensure they are large enough for the assay to distinguish them. Ideally, test at least 2 to 4 titration points in both directions until you reach the plateau(s). (Note that the lower plateau is not as important as the upper plateau).

To increase reproducibility and compensate for the inherent variability of bioassays, measure each titration point in 2 or 3 technical replicates. Measuring more titration points yields more reliable results, but practical considerations (cost, effort, time) may mean that a full set is not possible. Try to find a good compromise.

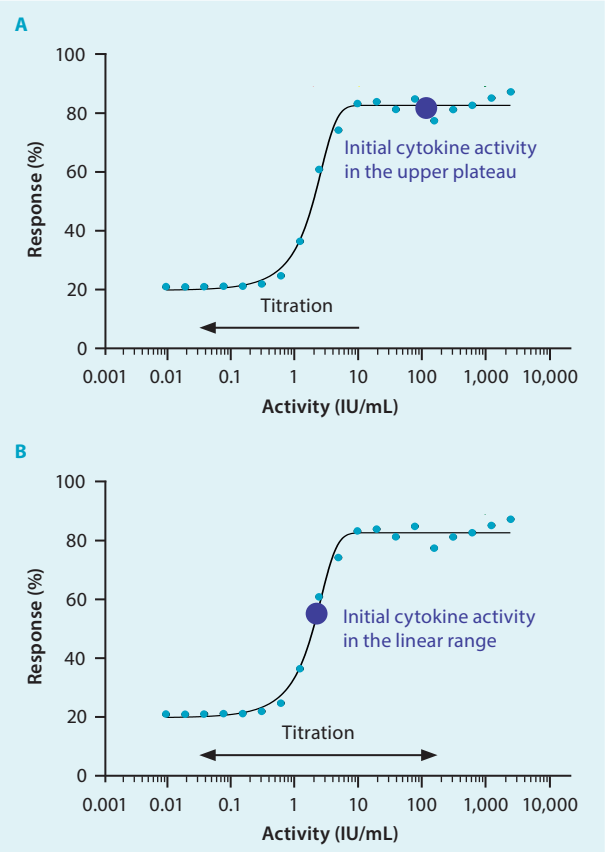

Figure 2: The dose-response curve and titration setup. If the initial cytokine activity is in the saturation phase (the upper plateau, A), the titrations should go down. If the initial cytokine activity is in the linear phase (B), the titrations should go up and down.

The next consideration is whether you are carrying out activity- or weight-based cytokine dosing (IU/mL vs. ng/mL). For successful side-by-side comparisons, the dosing must be in units. If the biological activity of the applied cytokine is known, use this formula for the conversion:

$$\text{Protocol concentration in IU/mL} = \frac{\text{Biological activity (IU/mg)} \times \text{Protocol concentration (ng/mL)}}{10^6}$$

If you do not know these parameters, we advise determining them experimentally. However, since the assay is the intended use case for the cytokine, it is probably well characterized.

What limits the application of side-by-side testing?

If any of the following apply, side-by-side testing is not reliable:

- The comparison is performed at saturating or sub-optimal concentrations.
- Too few repetitions are performed.
- The sensitivity or variability of the bioassay are unknown.
- Suboptimal cellular material is used.
- The tested items were compared in separate tests.
- The test system's response is affected by contaminants.

There are also unavoidable variations to consider. For example, outer wells often show higher variation. Testing samples multiple times and varying their position on the plate can compensate for this.

Examples

To compare the performance of two different cytokine products, plot the results of the titrations against each other. Cytokines with a better performance will yield a better response at a given dose.

Parameters are known

Here is a comparison between two cytokine products, A and B. The assay has been set up with a third cytokine product, C, to generate the parameters below:

- Cytokine activity usually used: 10,000 IU/mL
- Upper plateau for C starts at: 2,000 IU/mL
- Lower plateau for C ends at: 1 IU/mL

The aim is to compare multiple cytokine products to identify the one with the best performance. Based on the known activity for cytokine C, four titration steps for A and B will be carried out in triplicates:

- Titration steps for A and B are: 2, 20, 200, and 2,000 IU/mL

Results are shown in figure 3. Cytokine B elicits a response of ~35% of the maximum possible response at 2 IU/mL, while cytokine A only elicits a response of around 5%. At 20 IU/mL cytokine B elicits a response of ~65% of the maximum possible response, while cytokine A only elicits a response of around 30%. A similar pattern is visible at the other two titration points.

In conclusion, cytokine B showed an overall better performance than cytokine A (fig. 3).

The final decision for a specific cytokine product depends on price, purity, supply security, and other factors. However, this side-by-side comparison of performance indicates that cytokine B is better for this assay.

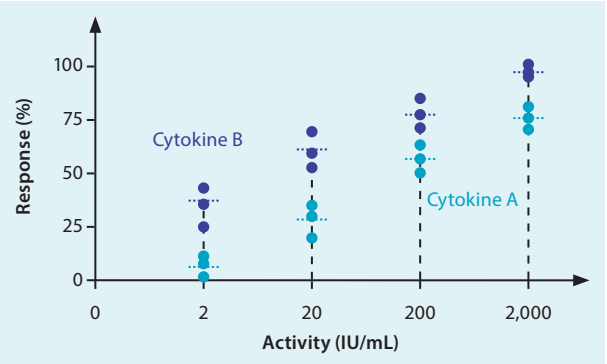

Figure 3: Results of the side-by-side comparison assay. Shown are the results for three identical experiments for cytokine A and B and the respective mean.

Parameters are not known

If the response of the assay system to the cytokine products is unknown, data from peer-reviewed literature data must be taken as the starting point for the titration. That means carrying out a broader titration. In most cases, this will be done with one cytokine product to establish the dose-response curve for the assay with this specific cytokine and to narrow down the test range for the subsequent comparison of multiple cytokine products. After generating these data through multiple assays, the cytokine products can be compared as described in the section above.

As an alternative, the broader titration can be carried out with multiple cytokine products in parallel. While this is more expensive due to the higher amount of cytokine required, this approach yields a comprehensive comparison between the cytokine products and saves time, as both the initial setup and comparison of the various cytokine products can be carried out in one series of experiments.

Conclusion

You should always test a cytokine product in the intended application to ensure clarity on how the supplier's activity values translate to the specific application. You are also advised to run side-by-side comparisons of cytokines from different suppliers to ensure that you invest in the most suitable cytokine for your application.

References

1. Meager, A. (2006) Methods 38(4): 237–252.
2. Meager, A. (1988) Dev. Biol. Stand. 69: 199–206.

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