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SCIENTIFIC

TECHNICAL NOTE

PIONEERING VACCINE TRIALS: UNVEILING THE SCIENCE BEHIND NEUTRALIZING ANTIBODY ASSESSMENT

Neutralizing antibodies (NAb) can be a critical component to assessing safety in therapeutic drug trials or efficacy in vaccine trials. This bulletin highlights the utility of using cell-based neutralizing antibody assays and provides insights on some best practices for industry use where high-throughput capacity and reliable reproducibility are required.

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Introduction

In the realm of public health, where the importance of viral vaccines continues to grow, large-scale vaccine trials stand as linchpins in safeguarding the well-being of millions, if not billions, worldwide. At the heart of evaluating vaccine efficacy and safety lies the indispensable Neutralizing Antibody (NAb) assay, particularly the biologically potent cell-based approaches.

The Utility of Cell-based NAb Assays

The presence of neutralizing antibodies to therapeutic compounds can have implications for the safety and efficacy of the product and assays designed to detect them are often crucial components of clinical trials for this reason (U.S. Food and Drug Administration, 2014). Cell-based NAbs are assays capable of detecting the presence of neutralizing antibodies *in vitro* and involve human matrix. As a result, NAbs are based on biological activity more akin to the potential scenario in the trial subject and thus have been generally preferred by regulators (Gupta et al. 2007; Wu et al. 2016). This approach is also used to assess the effectiveness of vaccines in eliciting antibody responses over time. Such approaches have particularly been used in recent years to prove that vaccines produce protective antibodies against SARS-CoV-2 (Chmielewska et al. 2021).

However, designing, validating, and executing these assays is no small feat. Enter Eurofins Viracor BioPharma Services, renowned for its extensive track record in providing not only high-quality but also high throughput NAb assays crucial for large vaccine trials. This intricate methodology involves designing and constructing suitable infectious reporter viruses, employing live cell culture, and devising a systematic approach to interpreting immunological responses from a living cell model. At the core of this lies thorough R&D efforts that produce accurate, sensitive, specific, and precise assays capable of being efficiently executed by multiple operators for use in high-volume clinical testing.

Methodology

Cell-based NAb assays entail a meticulous process wherein patient serum, potentially harboring neutralizing antibodies, is combined with the target virus and tested on cells susceptible to infection by the virus of interest (Figure 1a).

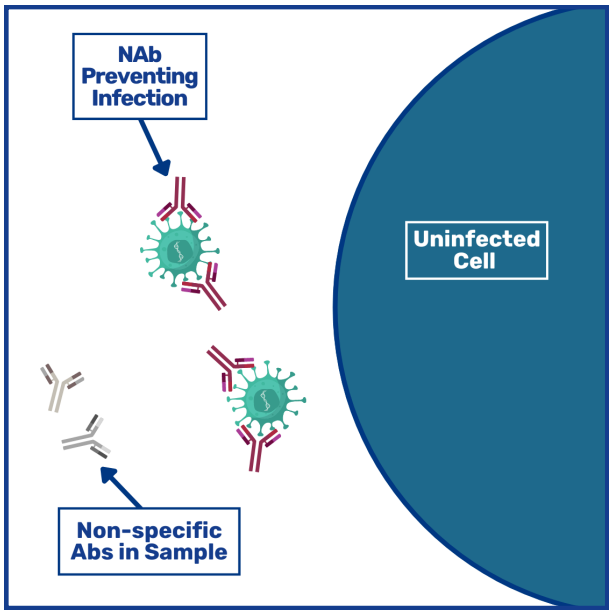

Figure 1a

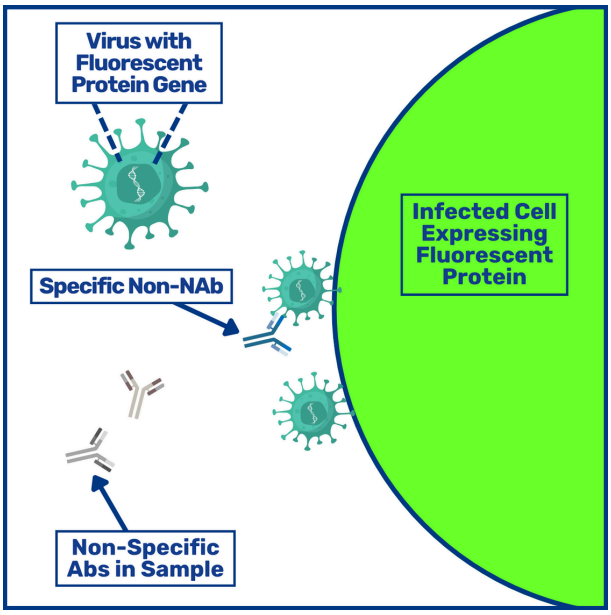

Figure 1b

The ability to appropriately detect infection is paramount to the success of this approach. A primary example is that of a vaccine-targeted virus engineered to encode for Green Fluorescent Protein (GFP) commonly chosen for versatility across various viruses and vaccine trials (Figure 1b).

A reliable means to quantify the presence of neutralizing antibodies in subject serum is by using a calculated plate-specific cutoff to determine titers of serially diluted samples, allowing reproducible longitudinal analysis over a study (Zielinska et al 2005). An example is shown in Figure 2.

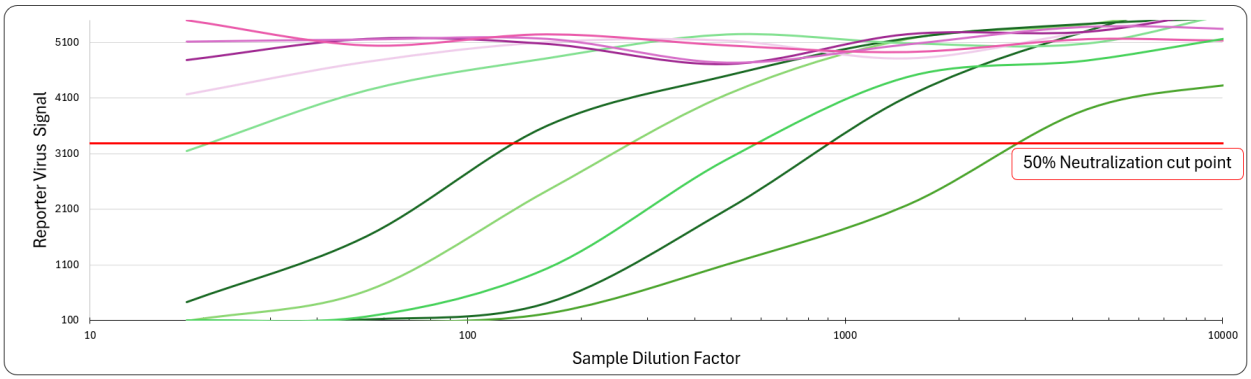

Figure 2. Graph depicting data from a cell-based NAb experiment including a fluorescent reporter across multiple serial dilutions. Each line represents serum samples with varying titers of neutralizing antibodies. The observed Reporter Virus Signal correlates with cell infection level which is inversely proportional to the level of neutralizing antibodies, with lower infection rates corresponding to higher levels of neutralizing antibodies. This is reported as a titer at the point at which it crosses the calculated 50% neutralizing antibody cut point. Positive samples are colored in green, negative samples are colored in purple.

Best Practices

A successful NAb assay hinges on several key technical aspects including cell culture techniques, reporter systems, and reliable high-throughput techniques. Eurofins Viracor BioPharma has demonstrated the use of direct seeding of cryopreserved (ready-to-plate) cells. This approach utilizes the same passage across the assay while greatly reducing variability, compared to traditional cell passage methods, and generally does not compromise cell vigor or response to infection (Table 1 & Figure 3).

Table 1

	Cultured Cells	Direct Plated Cells
	265	268
	331	300
	303	297
	266	274
	257	308
	267	310
	279	373
	230	304
	205	324
	287	306
	314	326
	263	348
	263	290
	290	319
	257	368
	245	265
Mean	270	311
Std.Dev	31	32
%CV	11%	10%
%Difference	13%	

Figure 3a

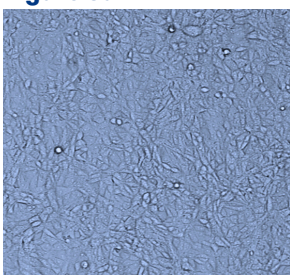

Figure 3b

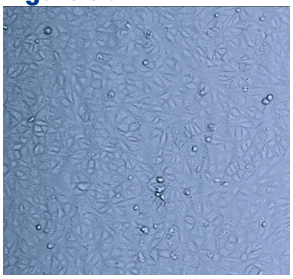

Table 1. Example data comparing fluorescent signal of wells of Vero cells either: cultured over three passages and plated following harvest (cultured cells); or plated from cell aliquots frozen in vapor phase liquid nitrogen and plated immediately following a thaw and wash to remove the freezing medium (direct plated). Cells were plated at equal densities per well, infected with identical concentrations of a fluorescent reporter virus, and incubated for 18 hours before analysis.

Figure 3. Example images of cultured cells (a) and direct plated cells (b).

Utilizing a quantitative infection detection system employing either reporter viruses expressing fluorescent proteins or luciferase, or the use of unmodified viruses combined with detection using stains or antibody-based labeling is essential. In development, the appropriate infection rate of the virus of interest or virus reporter should be determined and matched with the appropriate permissive cell line and finally be optimized to effectively show neutralization across an acceptable linear range. Maintaining bulk preparations for standardized supplies of critical reagents, particularly cell lines and reporter viruses, is crucial for accurate results.

Adopting reliable automated reading systems ensures consistency and rapidity across multiple runs while helping to ensure sustained quality that is easily scalable for high throughput capacity. Additionally, long lead times for procuring viral and cell materials can be mitigated by employing a modular and robust assay pipeline that supports the use of bulk materials. Lastly, standardized pipetting techniques and high throughput capabilities can be paramount to the success of conducting large-scale vaccine trials effectively.

Conclusion

In summary, Eurofins Viracor BioPharma's comprehensive approach to NAb assays not only underscores a commitment to advancing public health but also highlights the intricate interplay between cutting-edge technology and meticulous methodology in the realm of vaccine research. This culmination of efforts results in validated assays that are consistently reproducible with high levels of accuracy and precision that enable multiple sample batches per day. By catering to the diverse array of industry needs, Eurofins Viracor BioPharma facilitates swift progress in the pursuit of assessing vaccine safety and efficacy.

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