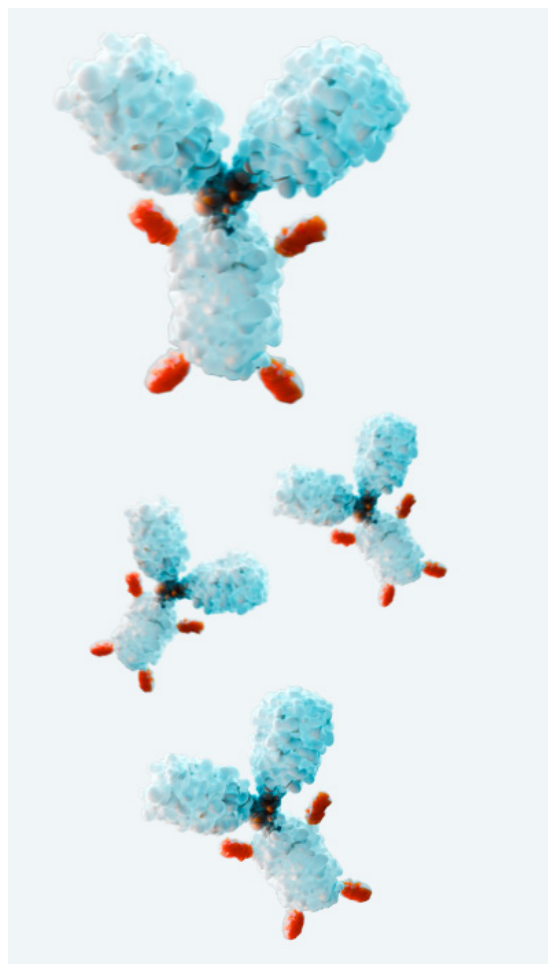


Reducing cycle time and sample volume by using Gyrolab® assays to measure PK/TK of an antibody-drug conjugate (ADC)

CASE STUDY

- Gyrolab® assays were rapidly developed to study the pharmacokinetic (PK) profile of an ADC *in vivo*
- The Gyrolab assays were run in parallel to reproducibly determine serum concentrations of total antibody and intact ADC
- The assays were run in unattended mode and required small volumes, saving precious reagents and sample



Summary

The therapeutic field of antibody-drug conjugate (ADC) drugs is growing and the complex composite nature of the ADC means an increased need for assays that can reliably detect both intact ADC and total antibody (Ab) in biological samples with a throughput matching the drug discovery timelines. In this case study researchers rapidly developed Gyrolab assays to reproducibly measure total Ab and intact ADC in serum from rats dosed with ADC for pharmacokinetic (PK) profiling in toxicology studies.

Background

ADCs are an expanding class of therapeutics that combine the efficacy of small molecule drugs with the specificity and targeting of monoclonal antibodies (mAbs). This is especially important in cancer treatments where the linking of a cytotoxic agent to a tumor-targeting antibody spare other tissue, thereby reducing unwanted side effects and increasing efficacy (Shastri *et al* 2023).

The composite nature of the ADCs, with both a chemical entity and a biomolecule, means challenges when studying the PK and toxicokinetic (TK) properties. In addition, the linker may be cleaved *in vivo*, leading to increased levels of free payload. It is therefore important to distinguish between ADC and free antibody as well as free drug (Wagh *et al* 2018).

Plate-based ligand-binding assays (LBA) such as enzyme-linked immunosorbent assays (ELISA) are widely used in ADC bioanalysis, but they often fall short in terms of sensitivity, throughput, and reproducibility. In particular, susceptibility to matrix interference, and the need for multiple assay formats put pressure on traditional methods to deliver reproducible results within constrained timelines. It is also critical to keep sample and reagent volumes as low as possible to save precious reagents and reduce number of animals needed for PK/TK studies. Many laboratories are for these reasons turning to automated immunoassay platforms, like the Gyrolab platform (Figure 1).

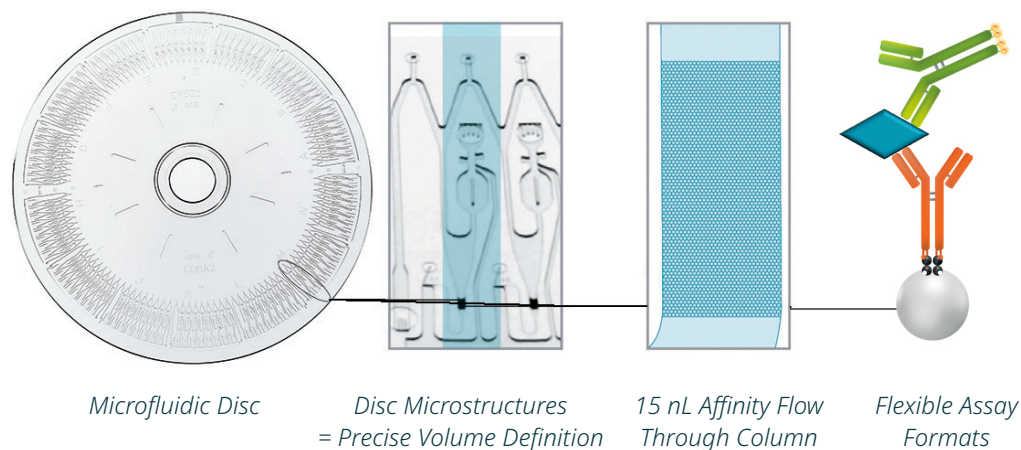


Figure 1: The Gyrolab immunoassay platform includes discs with miniaturized streptavidin-coated bead columns to tether biotinylated capture agents. Laser-induced fluorescence is used for detection. The flow-through method reduces the interaction time between sample and column, thereby reducing matrix effects and increasing sensitivity. The platform allows for multiple assay formats to be run in parallel.

PK/TK studies of ADC using Gyrolab platform

This case study describes how researchers from Pfizer developed Gyrolab ligand binding assays (LBAs) to study the toxicological profile of ADCs *in vivo*.

Goal

The goal was to develop a high throughput and streamlined process using the Gyrolab immunoassay platform to measure both total Ab and payloads of an ADC to support non-GLP toxicology studies.

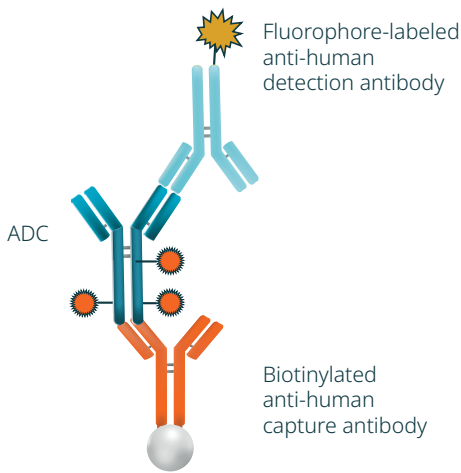
Challenge

The challenge was to rapidly develop assays to meet project deadlines with higher data quality and throughput with reduced reagent consumption.

Method

A sandwich assay format was used to detect intact ADC and total antibody (free and drug-conjugated) using in-house generated capture and detection antibodies (Figure 2). The Gyrolab platform was preferred to an ELISA format because of the wide concentration range (~100-50,000 ng/mL), the rapid assay development and small reagent volumes needed (Table 1).

Total antibody assay



Intact ADC assay

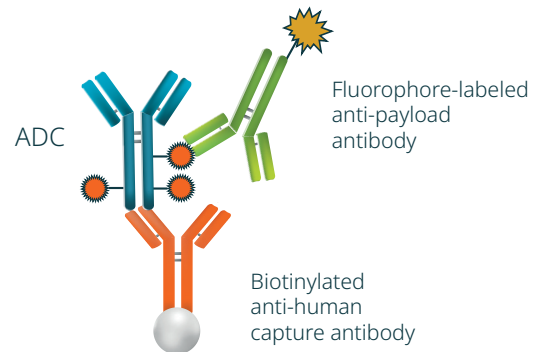


Figure 2: Setup of the Gyrolab PK assays.

Table 1: Gyrolab xP workstation demonstrated advantages over ELISA for assay development, run time, plus sample and reagent volumes, making it an ideal platform for bioanalysis of ADCs in preclinical and clinical PK/TK studies.

All data courtesy of Pfizer Inc.

	Gyrolab immunoassay	ELISA
Dynamic Range	~100 to 50,000 ng/mL in plasma	80 to 8,000 ng/mL in plasma
Assay Development	<2–4 days; 'Plug and Play'	2–3 weeks
Sample Volume Needed	< 10 μ L	25–100 μ L
Incurred Sample #	30/Gyrolab Bioaffy™ 200 CD	28/plate
Sample Setup Time	1 hr (less dilutions)	1 hr+
Run Time	~1 hr/CD (do O/N runs)	~5 hr/4 plate(s)
Anti-Payload Reagent as Capture	5 μ g/CD (50 μ L 1 well; 100 μ g/mL)	10 μ g (1 μ g/mL) or 2.4 μ g (1/2 area plate)
Anti-Payload Reagent as Detect	0.25 μ g/CD (50 μ L 1 well; 5 μ g/mL)	20 μ g (2 μ g/mL) or 5 μ g (1/2 area plate)
Other	Quick contact time; need high on-rate reagents. Less matrix interference.	Longer incubation times can accommodate lower affinity.
	1 st choice for LBA PK/TK	2 nd choice for LBA PK/TK

Results

The assay development was fast and the scientists developed GxP (good laboratory practice, good manufacturing practice) quality assays within 6 days. The lower matrix effect, because of the flow-through nature of the Gyrolab platform compared to the standard ELISA resulted in better data quality for the serum samples.

To study the PK/TK properties of the ADC *in vivo*, rats were dosed intravenously with the ADC. Serum samples were collected for 14 days and analyzed using the Gyrolab assays. The Gyrolab LBAs could clearly distinguish between PK of intact ADC and the naked antibody (Figure 3).

The rapid assay development and fast running of the assay, in an unattended manner (overnight) increased the throughput and shortened the turnaround times. They analyzed 240 samples per day and completed 1,200 sample analyses in 6 days.

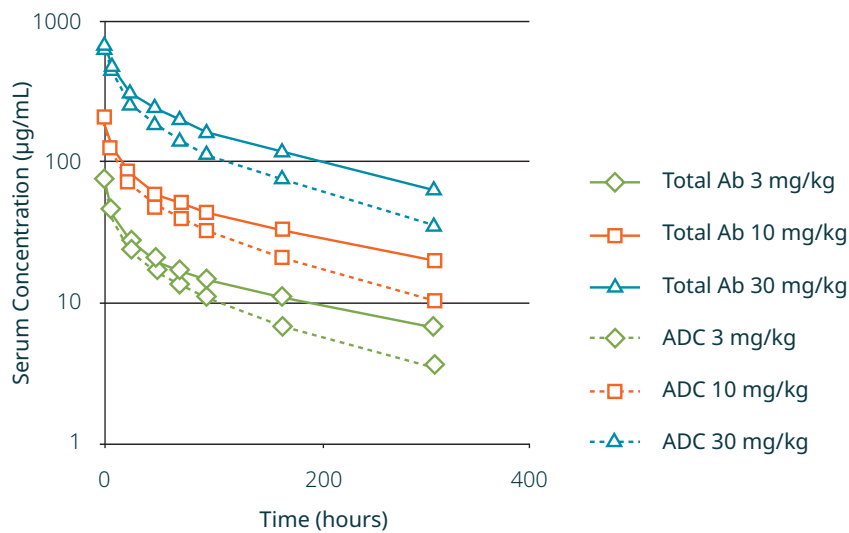


Figure 3: Gyrolab assays clearly distinguish between total Ab and intact ADC concentrations in rat serum after intravenous administration of ADC analyte at three different doses.

Summary

The Gyrolab platform is well suited for ADC PK studies. The assays developed were

Fast, <1h run time generating ~1000 datapoints per day with rapid method development.

Flexible, with simultaneous quantification of key ADC components, flexible to choice of reagents and reduced reagent consumption. High accuracy data in different biological matrices.

Convenient, more productivity with less hands-on time with overnight running.

Safe, enclosed system reducing direct operator contact with the ADC

Courtesy of Annette Wu, Senior Scientist, Pfizer, La Jolla

References

Shastry M, Gupta A, Chandarlapaty S, Young M, Powles T, Hamilton E (2023) Rise of Antibody-Drug Conjugates: The Present and Future. Am Soc Clin Oncol Educ Book 43, e390094 DOI:10.1200/EDBK_390094

Wagh A, Song H, Zeng M, Tao L, Das TK (2018). Challenges and new frontiers in analytical characterization of antibody-drug conjugates. MAbs, 10(2), 222

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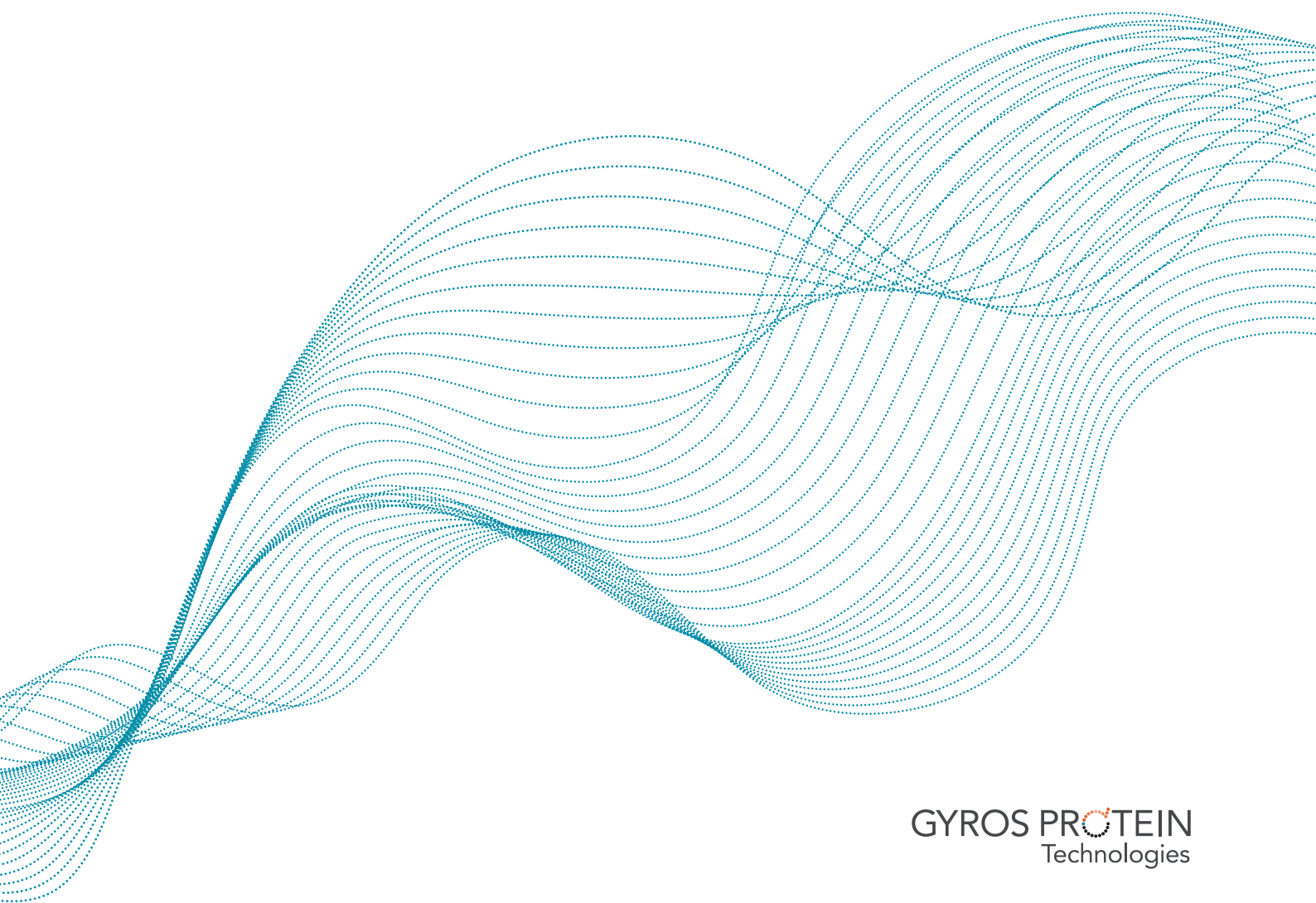
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