Quantitation of Antibody–Drug Conjugates

Application Note

D0020478

Introduction

Antibody–Drug Conjugates (ADCs) represent a novel type of therapeutic that uses the specificity of monoclonal antibodies to target a tumor-specific antigen and deliver a payload of small cytotoxic molecules to the tumor. Using Gyrolab™ xP workstation, a generic IgG assay and an assay to measure payloads of ADCs can be quickly developed in parallel.

Gyrolab xP workstation supports high productivity for ADC development in support of both toxicology (non-GLP) studies and subsequent regulated studies.

Efficient ADC assessment: screen hundreds of linker-payload combinations in vivo simultaneously.

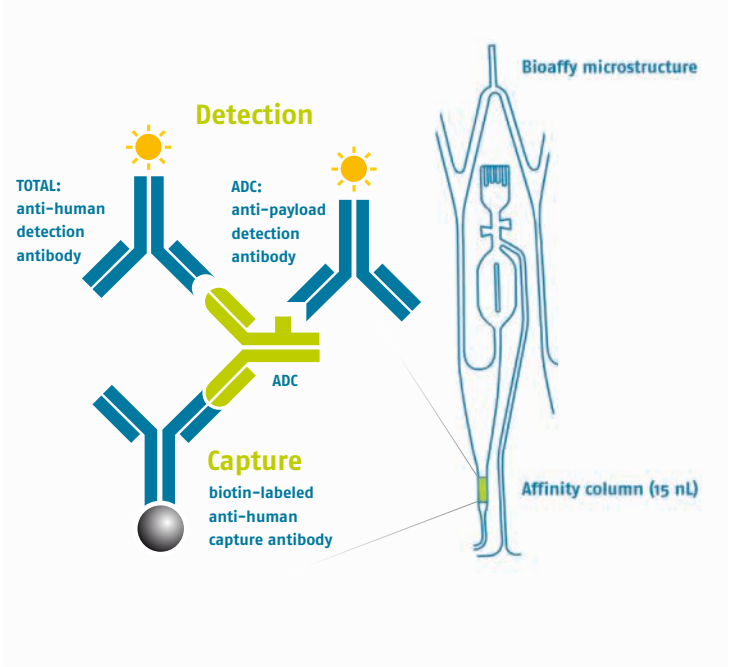
Less sample and fewer animals: Gyrolab xP workstation requires <10 µl/sample, permitting serial mouse sampling.

Rapid time to results and high throughput: Results in less than one hour for one CD. Five CD runs provide higher throughput and overnight run capability.

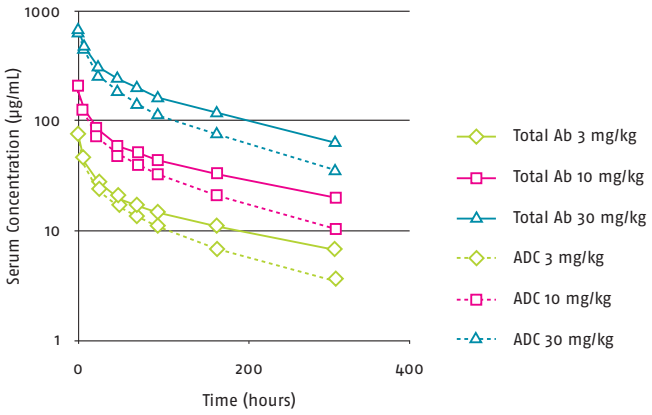
Broader dynamic range: Assays with 3–4 logs of dynamic range require less dilution.

Fewer resources: Automation and parallel processing minimize FTE resources.

Measuring total antibody and intact ADC



Intact ADC and total antibody in vivo



Samples were taken from rats dosed with ADC and naked antibody. The Gyrolab assays could clearly distinguish the pharmacokinetics of the intact ADC compared with the total antibody.

Nanoliter-scale immunoassays on Gyrolab xP workstation

Gyrolab xP workstation automates immunoassay workflows at the nanoliter scale in a compact disc (CD) format by integrating sample addition, incubation, washing and fluorescent detection. Centrifugal force, capillary action and hydrophobic barriers precisely control the parallel processing of up to 112 reactions in the Gyrolab Bioaffy 200 CD, providing results in under an hour.

The biotinylated capture antibody binds to streptavidin-coated beads in the affinity column. Gyrolab xP workstation transfers the sample to the CD followed by a detection antibody labeled with fluorophore. In this example, biotinylated anti-human IgG Fc mAb was used as a capture reagent. Total antibody was measured using anti-human kappa antibody labeled with Alexa Fluor 647 and ADC was measured using labeled anti-payload antibodies. A detector records the laser-induced fluorescence from each column.

The sample is quantitated using a standard curve. Results are evaluated using Gyrolab Evaluator, or exported to a LIMS. All software programs are designed for 21 CFR part 11-compliance, ensuring that assays can be developed and transferred in regulated environments.

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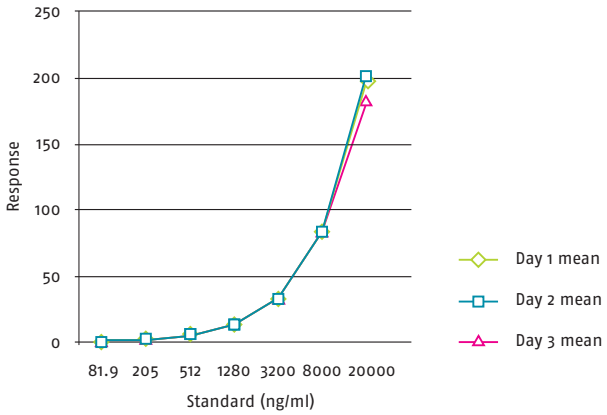
High reproducibility



Gyrolab xP workstation delivers standard curves that are highly reproducible from day to day, ensuring more robust data for optimization.

Comparison of ADC sample analysis

	Gyrolab	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 µl	25–100 µl
Incurred Sample #	30/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 µg/CD (50 µl 1 well; 100 µg/ml)	10 µg (1 µg/ml) or 2.4 µg (1/2 area plate)
Anti-Payload Reagent as Detect	0.25 µg/CD (50 µl 1 well; 5 µg/ml)	20 µg (2 µg/ml) or 5 µg (1/2 area plate)
Other	Quick contact time; need high on-rate reagents. Less matrix interference.	Longer incubation times can accommodate lower affinity.
	1st choice for LBA PK/TK	2nd choice for LBA PK/TK

Gyrolab xP workstation has several advantages over ELISA, making it an ideal platform for bioanalysis of ADCs in Pharmacokinetics/ Toxicokinetics.

All data courtesy of Pfizer Inc.

Conclusions

Gyros technology delivers high precision results over a broad dynamic range. The high reproducibility ensures an effective optimization process. Nanoliter-scale assays ensure low reagent and sample consumption. Finally, automation, ease of use and a fast assay turnaround on Gyrolab xP workstation save analyst time, increasing productivity and efficiency for time critical workflows.

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