DEVELOPMENT OF A mAb GENERIC PK ASSAY
Exploratory Comparison of the ELISA, MSD and NULISA Technologies

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CONTEXT

A **generic PK** (PharmacoKinetics) assay is an assay designed to detect and quantify multiple monoclonal antibodies (mAb) in animal matrices - without having to redevelop a method for each candidate.

OBJECTIVE

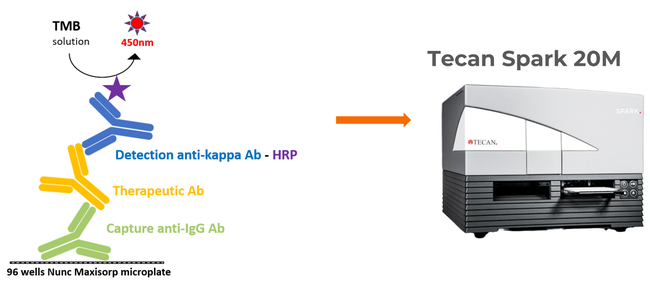
Comparison of **NULISA** ARGO™ HT platform from Alamar Biosciences with **ELISA** (Tecan Spark 20M) and Meso Scale Discovery **MSD** QuickPlex SQ 120 instruments to assess the quantification range, sensitivity, and the ability to limit sample dilutions in a **generic PK assay**.

MATERIALS & METHODS

Technologies	ELISA | MSD | NULISA
Drugs	Rituximab and Cetuximab (chimeric IgG1). Cetuximab has not been evaluated on NULISA.
Matrix	Non-human primate (NHP) serum
MRD	100
Principle	Anti-IgG capture and anti-κ light chain detection.
Antibodies	Identical across the 3 platforms.
Status	Exploratory assays. Some results are based on a limited number of runs.

COMPARISON OF 3 TECHNOLOGIES

I. ELISA

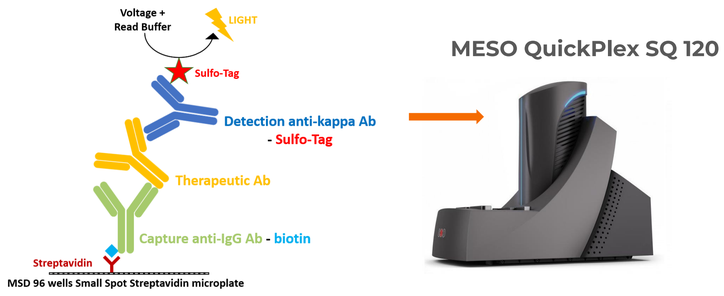


Method optimization

Standard ELISA method with optimization of :

- Concentrations of capture and detection antibodies
- MRD
- Blocking buffers
- Sample diluents

II. MSD



Method optimization

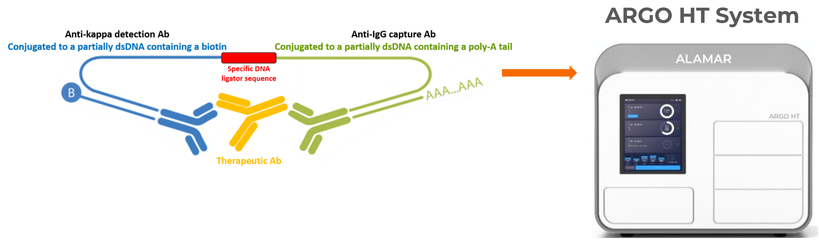
2 formats tested:

- Capture Anti-IgG-Biotin / Detection Anti-Kappa Light chain-SulfoTag ✓
- Capture Anti-Kappa Light chain-Biotin / Detection Anti-IgG SulfoTag

3 plate types tested:

- MSD GOLD 96-well **Small Spot Streptavidin** SECTOR Plate ✓
- MSD GOLD 96-well **Streptavidin** SECTOR Plate
- 96-Well 1-Spot SECTOR Plate (**standard plate**)

III. NULISA



Method optimization

Assay buffer

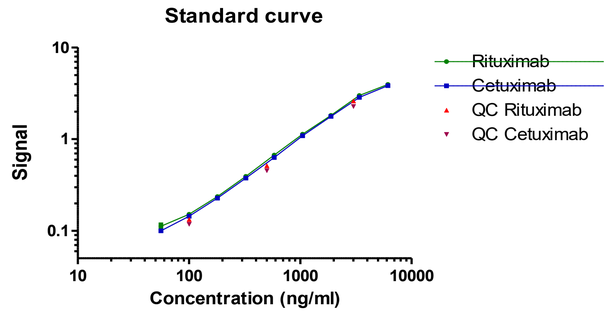
- AB0 (Alamar #801353)
- AB0 + 0.05% Tween 20 + 1% BSA
- PBS + 0.05% Tween 20 + 1% BSA
- PBS + 0.05% Tween 20 + 1% Casein
- Starting block (Thermo #37539)
- Superblock (Thermo #37515) ✓

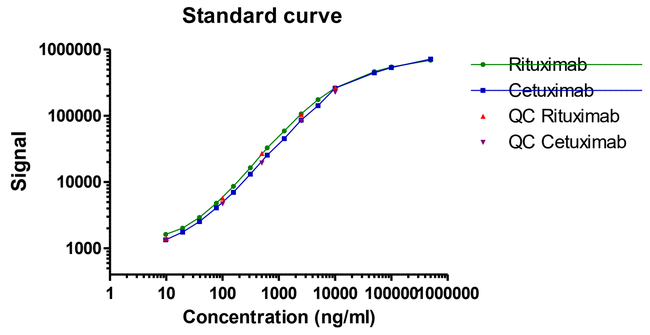
Capture & detection antibody concentration

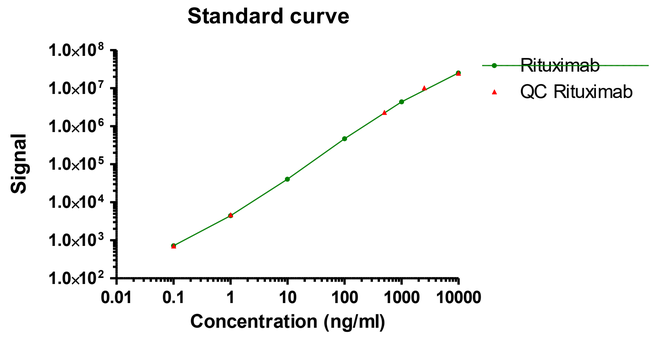
- Capture 100pM / detection 100pM ✓
- Capture 50pM / detection 100pM

Sample Diluent

- Alamar Sample Diluent SD (Alamar #801352)
- PBS + 0.05% Tween 20 + 1% BSA
- PBS + 0.05% Tween 20 + 1% Casein
- Starting block (Thermo #37539)
- Superblock (Thermo #37515) ✓







	ELISA	MSD	NULISA
Similarity of results between mAbs	Similar for Rituximab and Cetuximab	Similar for Rituximab and Cetuximab	N/A (Cetuximab not evaluated)
Detection range in NHP serum (ng/mL)	**~100 to 6,000**	**~20 to 100,000**	**~0.1 to 10,000**
Performance (Cal. and QC)		**S/N > 2; 80 < %ACC < 120; %CV < 20**	

THE APPROPRIATE TECHNOLOGY FIT FOR THE CONTEXT-OF-USE (CoU) : STRENGTHS, TRADE-OFFS & CAUTIONS

I. Technology comparison

	ELISA	MSD	NULISA
Upper quantification range (ng/ml)	6 000	50 000	10 000
Lower quantification range (ng/ml)	100	40	0.1
Dynamic range	1.8 log	3.7 log	5 log
Hook effect	Not studied	Not observed	Observed
Hands-on time	6h	6h	3h
Throughput/run	2 plates	2 plates	1 plate
Throughput/day/system	>6 plates*	>6 plates*	2 plates
Cost	$	$$	$$$
Need for antibody labeling	No	Yes	Yes
Points to watch out for	BSA batch	BSA batch	Variation on blanks
Best suited for	Limited budget	High throughput	Wide dynamic range High sensitivity

* Reading device: daily throughput depends on the number of operators.

II. Limitations and considerations

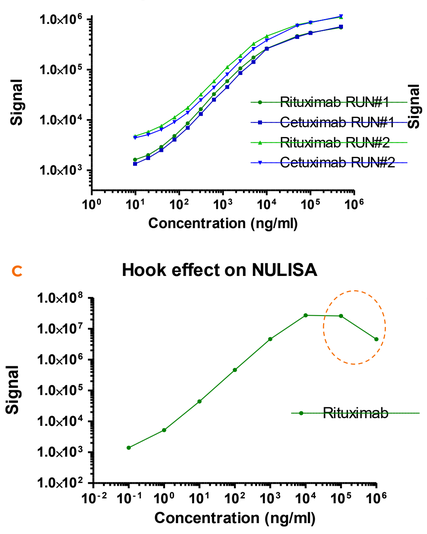
Signal variations between runs

- **BSA batch differences** could explain this signal variation (A)
- **Ig-related interference:** on all three platforms, be aware that the various buffers may contain IgG, which can interfere with the assay and cause signal variations between tests (B)

On NULISA

Watch for a **hook effect** at high concentrations and variations in the blanks (C)

Preliminary results: *methods not validated; data are still insufficient to conclude on their robustness and general practical value.*



CONCLUSION

- Feasibility of generic PK assay demonstrated on the **NULISA ARGO HT platform**
- Dynamic range & sensitivity: **NULISA > MSD > ELISA**
- Dynamic range limited by the **hook effect** on NULISA
- Caution regarding **IgG content** in assay buffer/diluent
- Ultimately, the best platform depends on the **project's needs (CoU):** cost, throughput, sensitivity, and range required