MORGAN EVANS – SENIOR SCIENTIFIC ADVISOR

Can technology choice make your data SPARCL?

QUALITY INNOVATION INTEGRITY

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AGENDA

01
An introduction to the background literature that inspired this work

02
Tocilizumab Case Studies: Critical Reagents and Methods.

03
Data, Data, Data!

04
Lessons learned and the future perspective.

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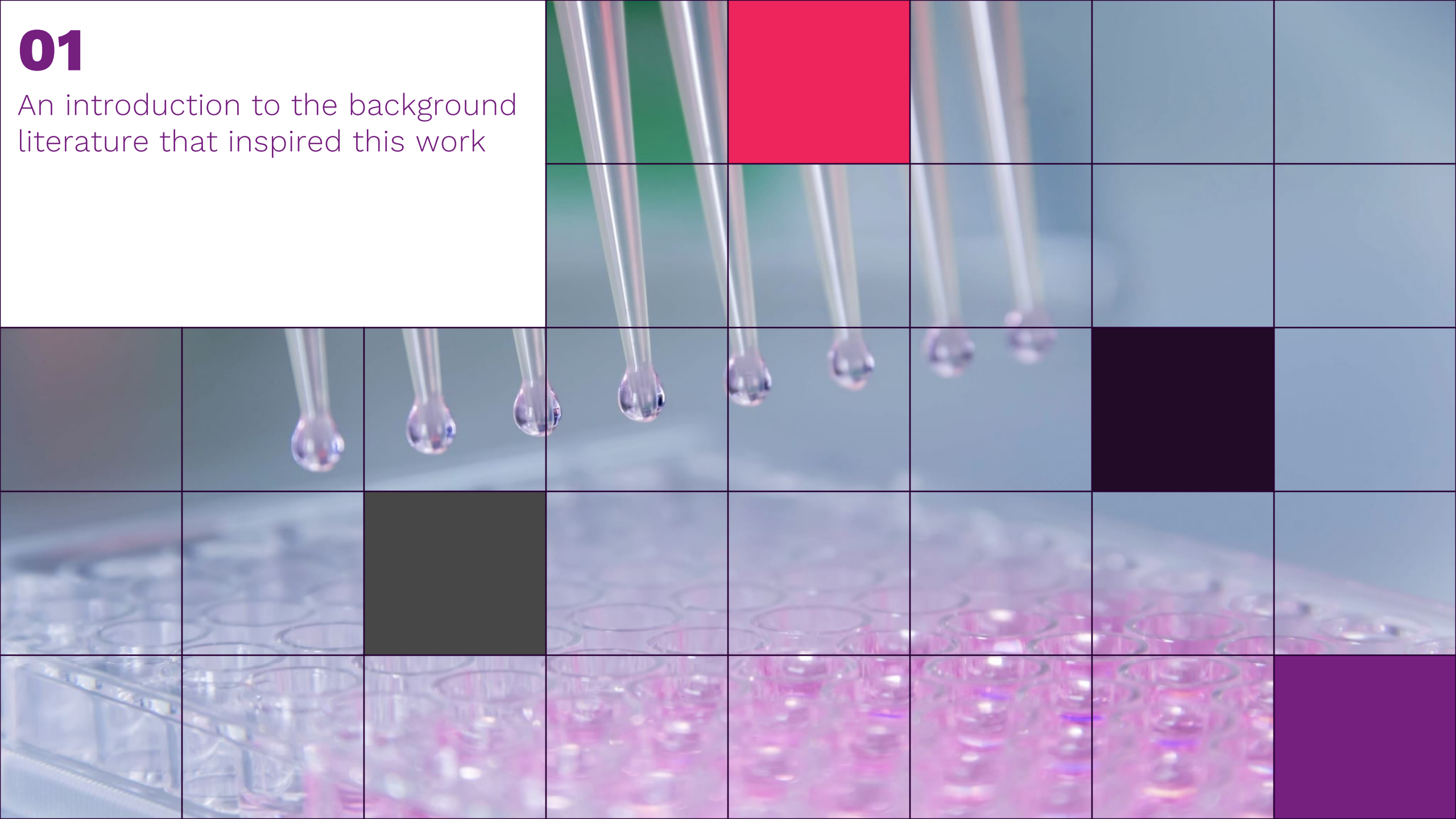

01 - BACKGROUND

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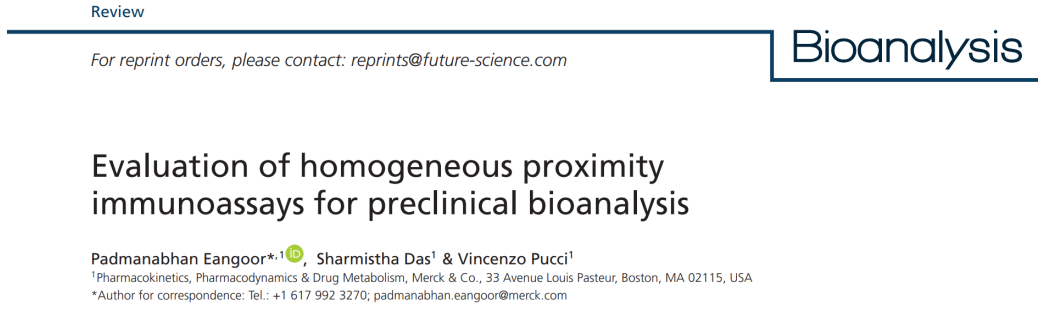

Review

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Bioanalysis

Evaluation of homogeneous proximity immunoassays for preclinical bioanalysis

Padmanabhan Eangoor*,[1], Sharmistha Das[1] & Vincenzo Pucci[1]
[1]Pharmacokinetics, Pharmacodynamics & Drug Metabolism, Merck & Co., 33 Avenue Louis Pasteur, Boston, MA 02115, USA
*Author for correspondence: Tel.: +1 617 992 3270; padmanabhan.eangoor@merck.com

Information:

MSD, Gyrolab®, HTRF, AlphaLISA®, and SPARCL™ technologies included.

Evaluation of IgG, IL-8 and Insulin assays.

Parameter	MSD	Gyrolab®	SPARCL™
Assay Time (Hrs)	4-6	1-2	1
Sensitivity	++++	++++	+++
Dynamic Range	3-4	3-4	3-4

Table modified from: Eangoor, P., Das, S., Pucci, V. (2020). Evaluation of homogeneous proximity immunoassays for preclinical bioanalysis. Bioanalysis, 12(24), 1757-1766. DOI: 10.4155/bio-2020-0258.

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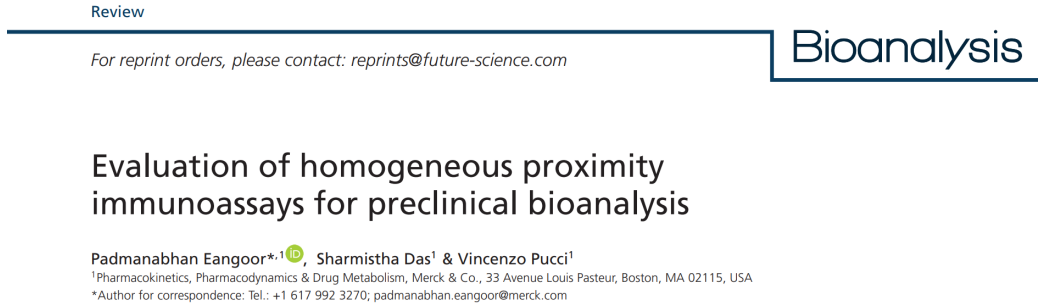

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The opportunity:

Assessment of the MSD, Gyrolab, and SPARCL platforms.

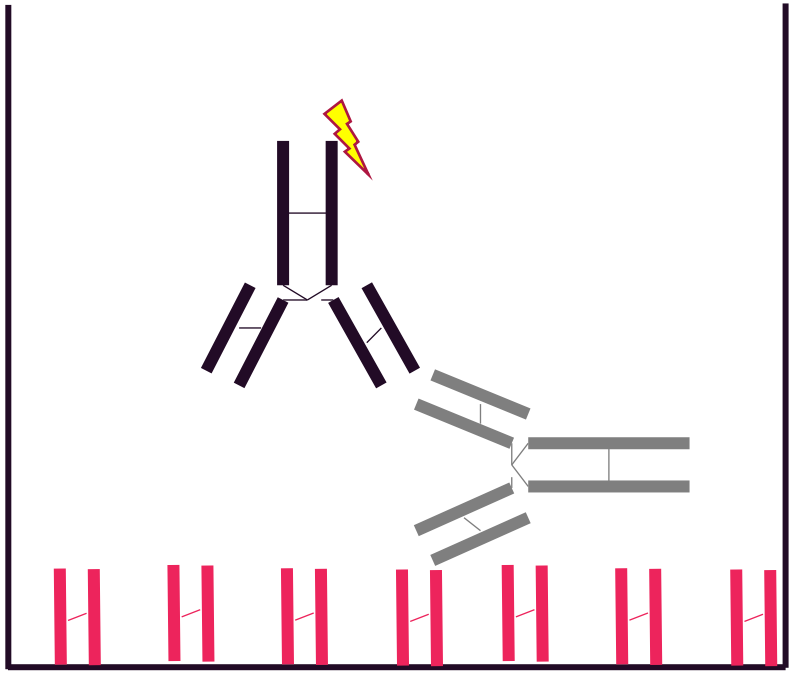
Utilising the same reference material and critical reagents such as capture and detection antibodies.

Table modified from: Eangoor, P., Das, S., Pucci, V. (2020). Evaluation of homogeneous proximity immunoassays for preclinical bioanalysis. Bioanalysis, 12(24), 1757-1766. DOI: 10.4155/bio-2020-0258.

02

Tocilizumab Case Studies: Critical Reagents and Methods

PK ASSAY FOR THE DETERMINATION OF TOCILIZUMAB IN HUMAN SERUM



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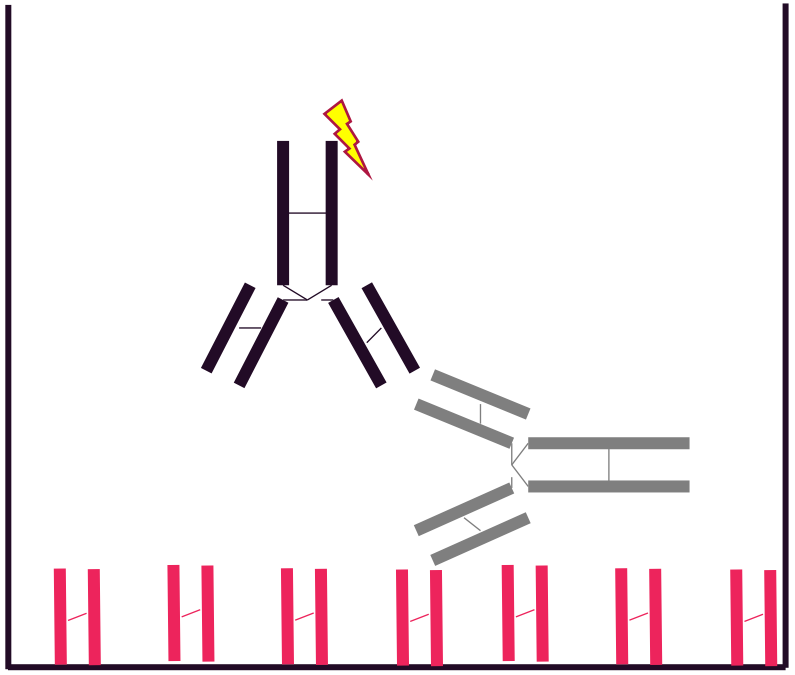
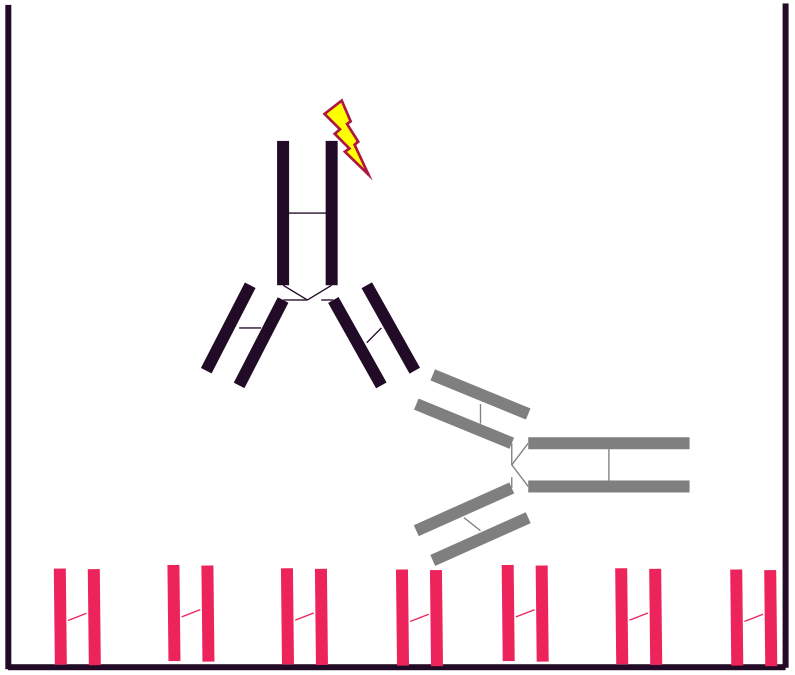


BioRad Clone, AbD21362

Tocilizumab

BioRad Clone, AbD21338_hIgG1

PK ASSAY FOR THE DETERMINATION OF TOCILIZUMAB IN HUMAN SERUM



BioRad Clone, AbD21362

Tocilizumab

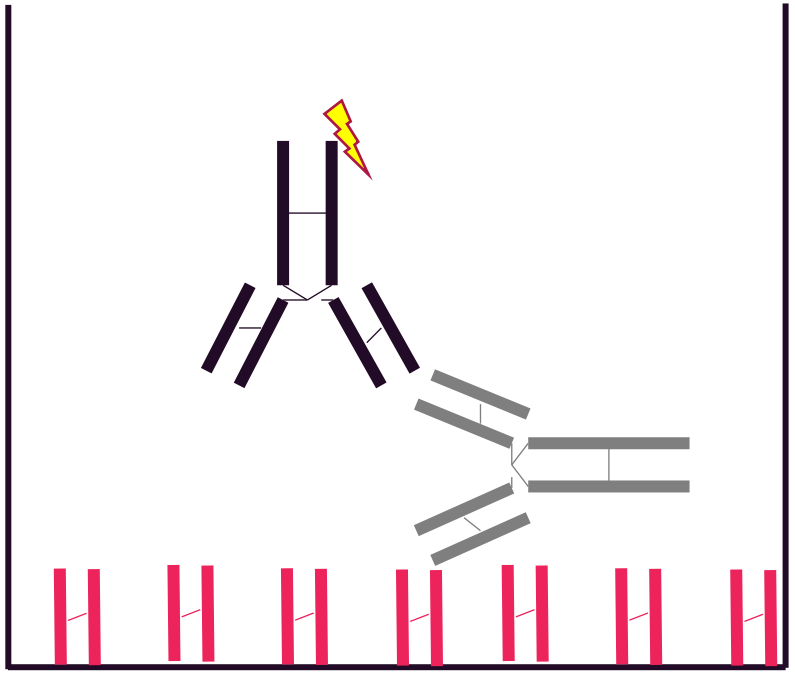
BioRad Clone, AbD21338_hIgG1

MSD: SulfoTag™

Gyrolab: Biotin & Alexafluor

SPARCL: HRP & Acridan

PK ASSAY FOR THE DETERMINATION OF TOCILIZUMAB IN HUMAN SERUM



BioRad Clone, AbD21362

Tocilizumab

BioRad Clone, AbD21338_hIgG1

MSD: SulfoTag™

Gyrolab: Biotin & Alexafluor

SPARCL: HRP & Acridan

- Bridging ELISA
- Varying labels for capture and detection reagent
- Tocilizumab was US drug with varying lot numbers

MSD

GYROLAB

SPARCL

Coating Incubation	
BioRad Clone, AbD21362	1 µg/mL, Overnight

Plate Blocking	
3% BSA in PBS	1-2 Hours

Sample Incubation	
MRD = 1 in 10	2 Hours

Detection Incubation	
BioRad Clone, AbD21338_hIgG1 (SulfoTag)	0.5 µg/mL, 1 Hour

Plate Reading
Read Buffer T 2X

Image: MesoScale Discovery. (2023). How does electrochemiluminescence work? [Digital image]. Mesoscale.com. Retrieved from https://www.mesoscale.com/en/technical_resources/our_technology/ecl

Prepare and Load Samples	
Rexxip H	MRD = 1 in 10

Prepare Capture Solution	
Capture: BioRad Clone, AbD21362 (Biotinylated)	350 nM, Bioaffy Bump Liquid

Prepare Detection Incubation	
Detection: BioRad Clone, AbD21338_hIgG1 (AlexaFluor)	1.67 nM, Rexxip F

Analysis	
Bioaffy 200 CD	Capture-Analyte-Detect (1% PMT)

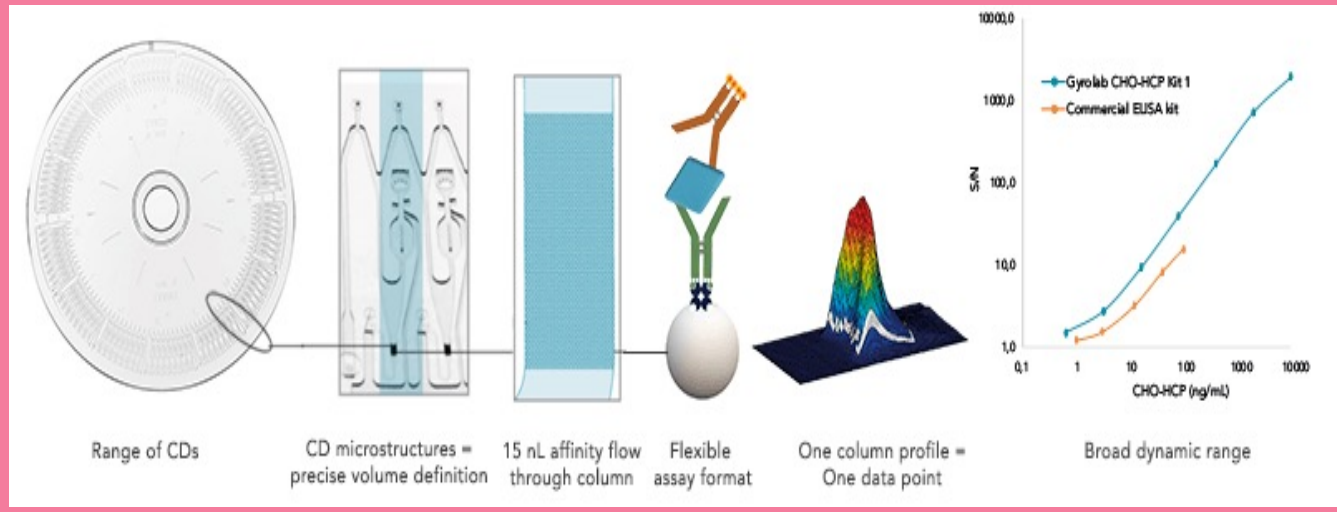


Image: Gyros Protein Technologies. (2023). One microstructure – One data point – No cross talk [Digital image]. Gyrosproteintechnologies.com. Retrieved from https://www.gyrosproteintechnologies.com/immunoassays/gyrolab-cds-nanoliter-precision

Prepare and Load Samples	
0.1% BSA in PBS	MRD = 1 in 10

Prepare and Load Master Mix		
Capture: BioRad Clone, AbD21362 (HRP)	Detection: BioRad Clone, AbD21338_hIgG1 (Acridan)	3 µg/mL, 0.1% BSA in PBS

Incubation	
Room Temperature	30 minutes

Load Background Reducing Agent	
Neat Agent	10% (v/v)

Analysis	
Inject Trigger Solution, 80 µL, 350 µL/s	50 Reads, Integrate 0.02s

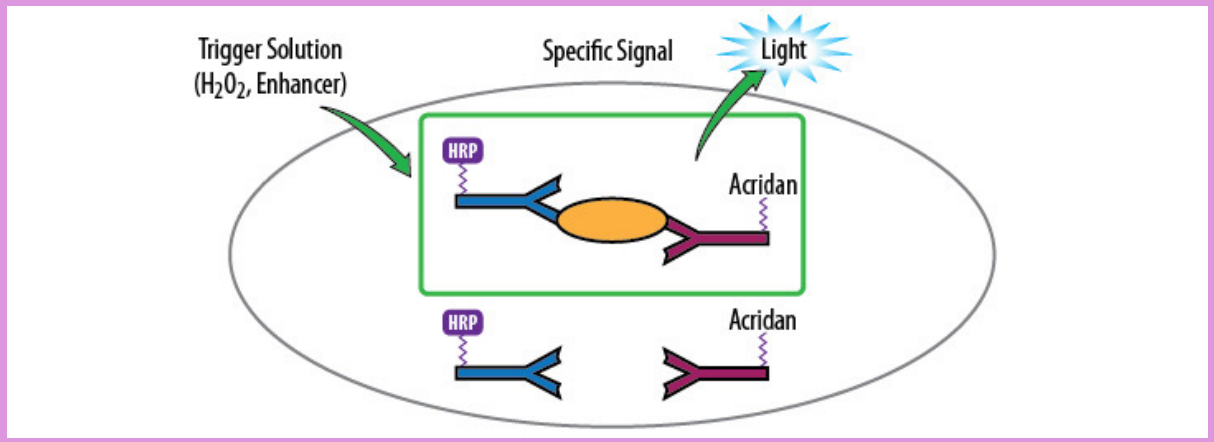
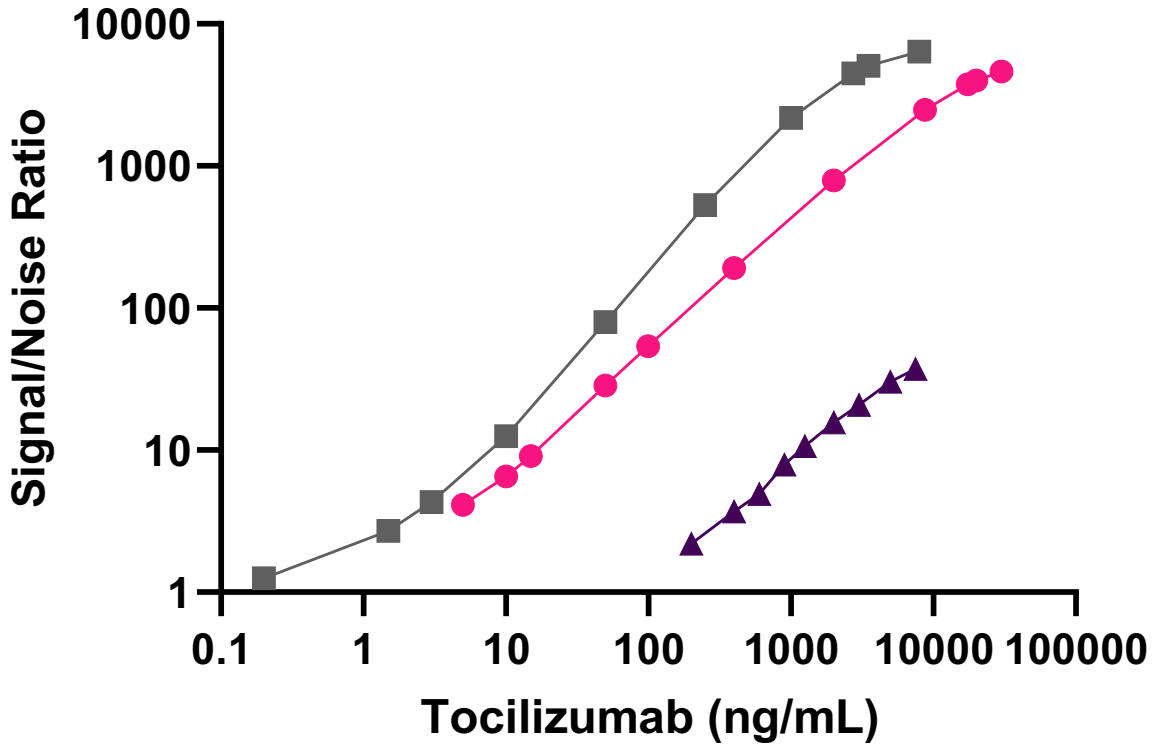


Image: Lumigen. (2022). Figure 2: A simplified mechanistic scheme of SPARCL technology [Digital image]. Lumigen.com. Retrieved from http://www.lumigen.com/products/elisa/lumigen-sparcl

03

Data, Data, Data!

RANGE AND SENSITIVITY



Gyrolab
MSD
SPARCL

Data normalised to signal to noise due to differing units.

MSD: RLU
Gyrolab: RFU
SPARCL: RLU(AUC)

Range (LLOQ-ULOQ):

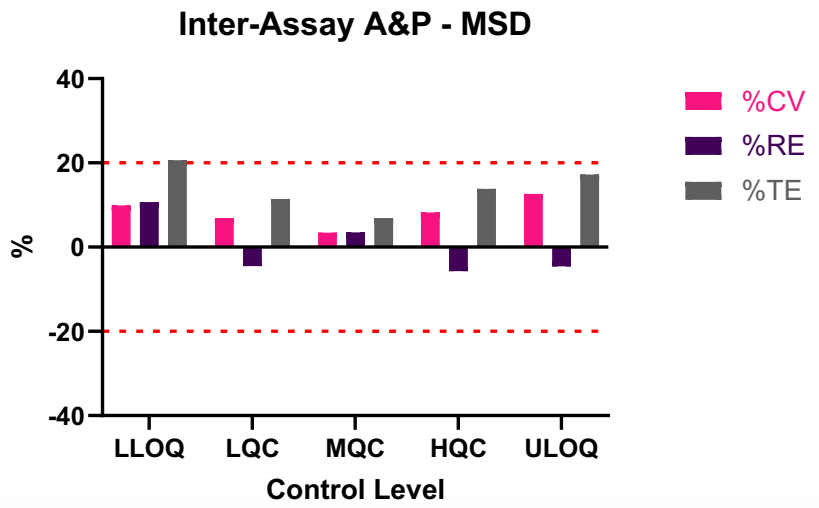
MSD: 1.50 – 3,500 ng/mL

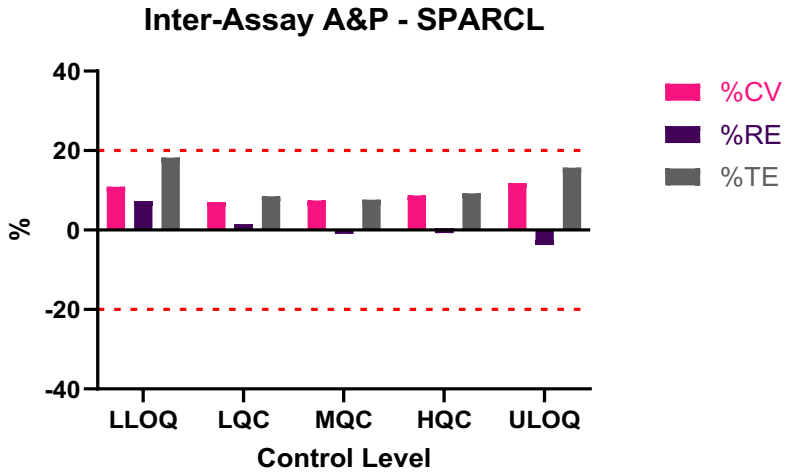
Gyrolab: 15.0 – 20,000 ng/mL

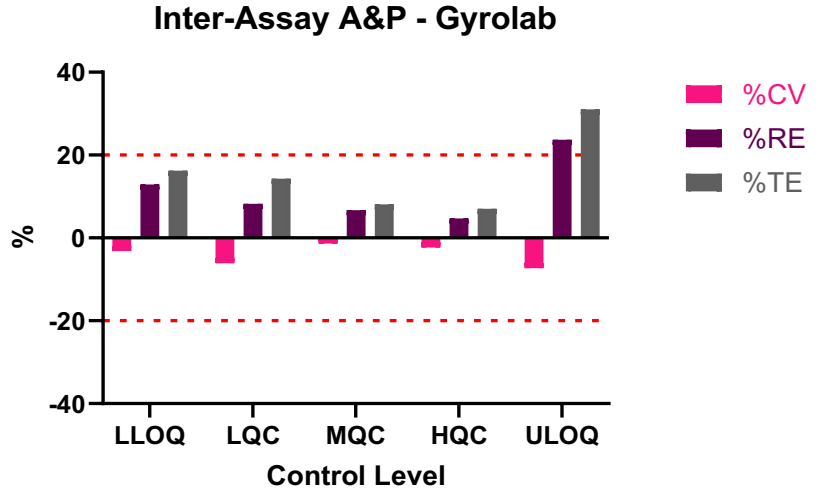
SPARCL: 400 – 7,500 ng/mL

INTER-ASSAY A&P

INTER-ASSAY A&P

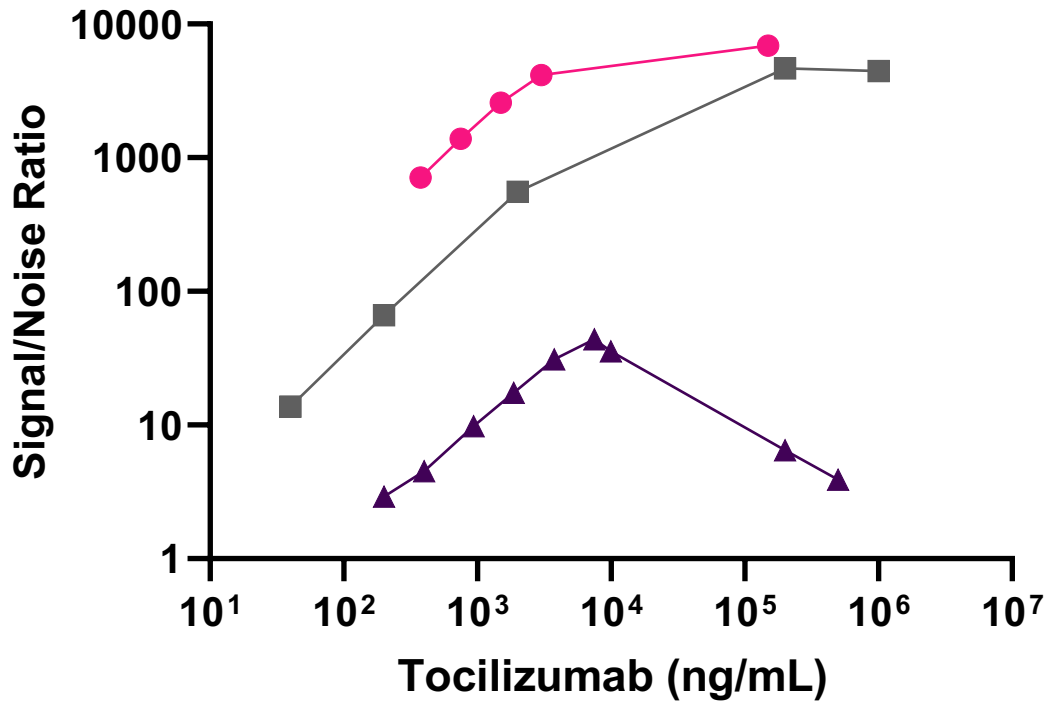






- All assay met A&P criteria across their range
- Gyrolab demonstrated poor recovery at the ULOQ.
- MSD & SPARCL demonstrated increased TE in the low range.
- SPARCL had excellent recovery QCL-QCH

PROZONE/DILUTIONAL LINEARITY ASSESSMENT



MSD
Gyrolab
SPARCL

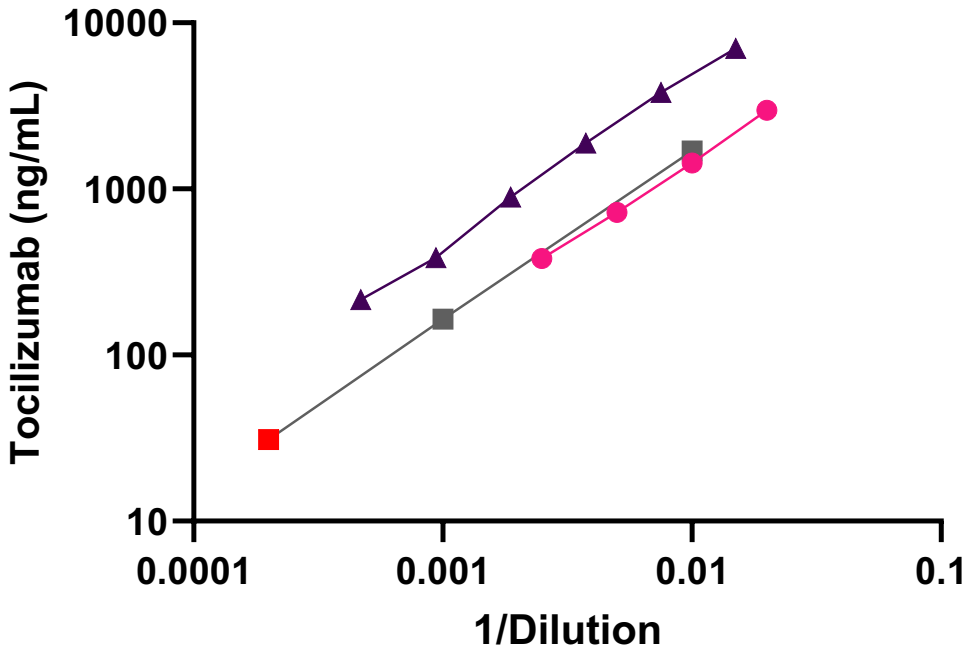
Data normalised to signal to noise due to differing units.

MSD: RLU
Gyrolab: RFU
SPARCL: RLU(AUC)

Prozone Observed:

MSD: No
Gyrolab: No
SPARCL: Yes, >7500 ng/mL

PROZONE/DILUTIONAL LINEARITY ASSESSMENT



MSD
Gyrolab (Red = Failure)
SPARCL

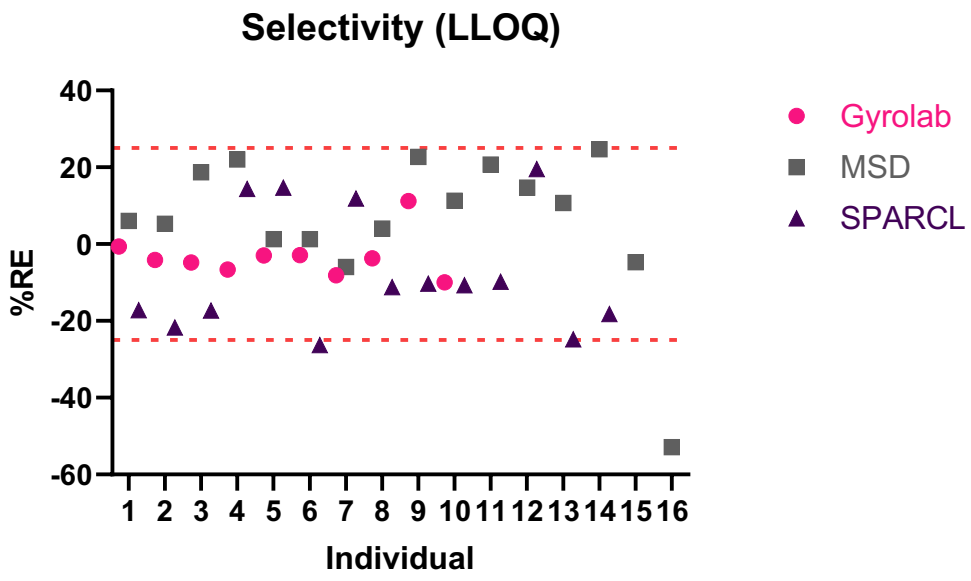
Data normalised to signal to noise due to differing units.

MSD: RLU
Gyrolab: RFU
SPARCL: RLU(AUC)

Dilutional Linearity was observed in all assays.

MSD: 1 in 400
Gyrolab: 1 in 1000
SPARCL: 1 in 1250

SELECTIVITY



Individual serum samples tested blank and at LLOQ.

No Individuals tested:

- MSD: 16
- Gyrolab: 10
- SPARCL: 14

Level	MSD	Gyrolab®	SPARCL™
Blank	100%	100%	100%
LLOQ	94%	100%	93%

04

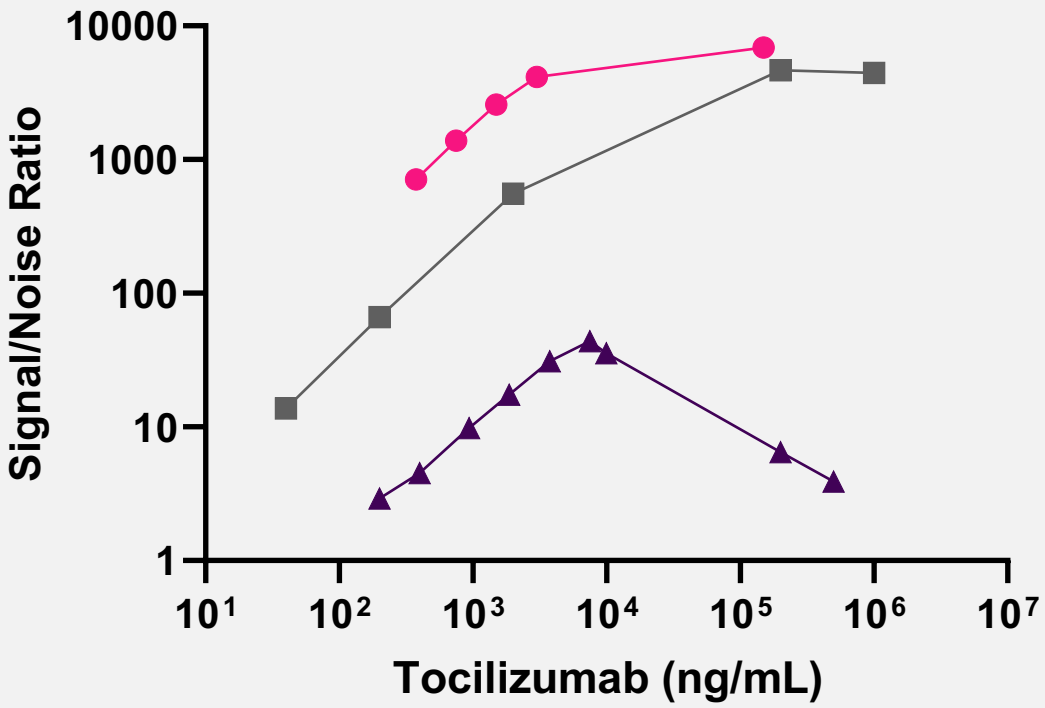
Lessons learned and the future perspective.

Parameter	MSD	Gyrolab	SPARCL
Range (ng/mL)	1.50 – 3500	15.0 - 20,000	400 – 7500
MRD	1 in 10	1 in 10	1 in 10
A&P	Pass	Pass	Pass
Prozone	No	No	**Extreme**
Dilutional Linearity	1 in 400	1 in 1000	1 in 1250
Selectivity	Pass	Pass	Pass

TECHNOLOGICAL
OPERATIONAL
FUTURE
PERSPECTIVE

OPERATIONAL FUTURE PERSPECTIVE

Parameter	MSD	Gyrolab	SPARCL
Run Time	5 Hours	2 Hours	1 Hour
No. Samples / Plate	32	40	32
No. Plates / Day	3	5*	12
Total Samples / Day**	96	200	384
*Utilising Gyrolab Xplore Model			
**Single Analyst			



Future SPARCL PK work:

- Re-optimisation of master mix reagents and concentrations
- Re-optimisation of background reducing agent.

Future considerations:

- SPARCL technology and qualitative assays (ADA)
- Impact of automation on throughput.

FUTURE PERSPECTIVE

Technology choice matters…

- MSD gives best sensitivity
- Gyrolab gives best dynamic range and least impact from matrix effects
- SPARCL gives the highest throughput but was restricted by prozone effect.

… but it must be matched with the critical reagents

- Critical reagent key quality attributes must be matched with the technology of interest to ensure the most appropriately designed assay.

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Any questions?

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