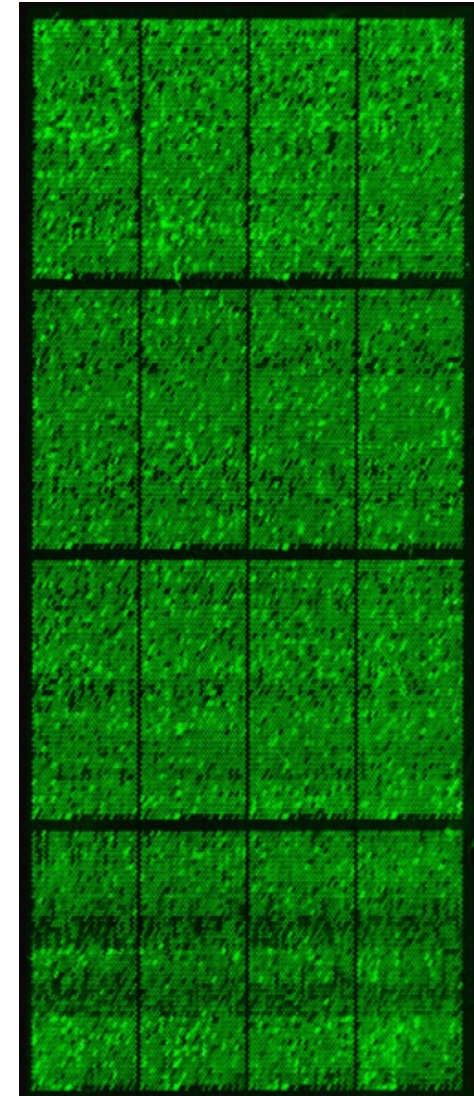
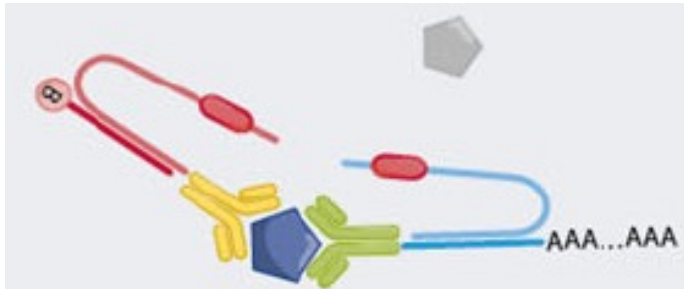
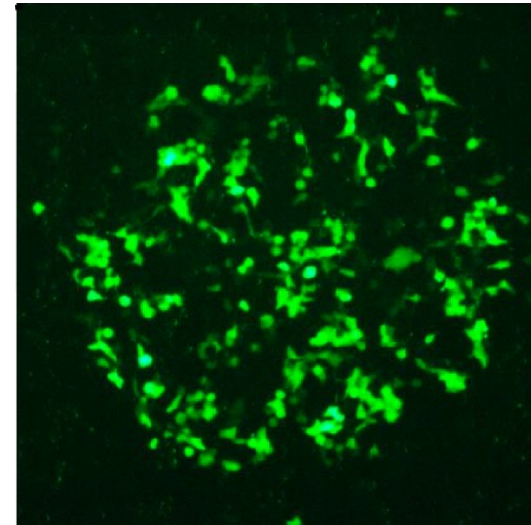
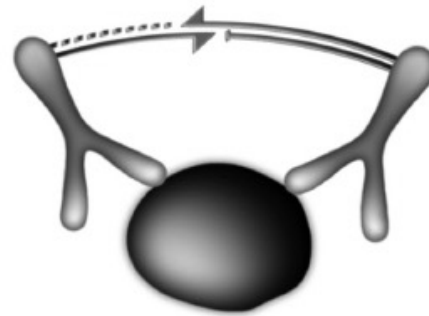
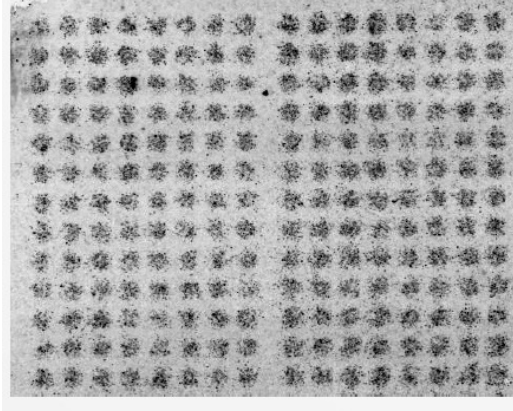


Updates on Assay Platforms for Serodiagnosis in NMOSD: Current Needs

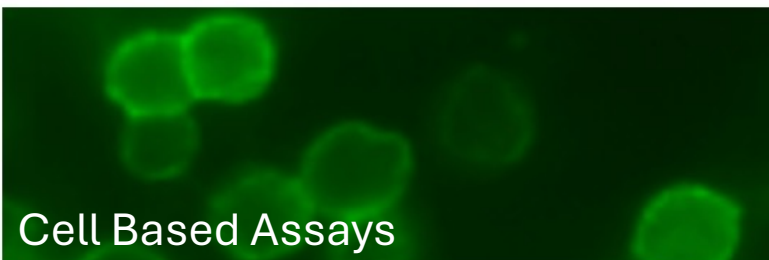
3rd August 2024



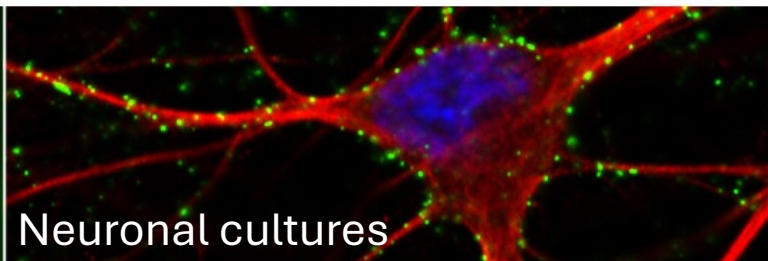
Patrick Waters PhD FIBMS FRCPath
Associate Professor, Co-Director Oxford Autoimmune Neurology Diagnostic Laboratory
University of Oxford

Disclosures

Patrick Waters is a named inventor on patents for antibody assays and has received royalties. He has received honoraria from Biogen Idec, Mereo Biopharma, Retrogenix, University of British Colombia, Euroimmun AG, UCB and Alexion; travel grants from the Guthy-Jackson Charitable Foundation; and research funding from Euroimmun AG. Oxford Autoimmune Neurology Diagnostic Laboratories performs live cell-based antibody assays.



Cell Based Assays



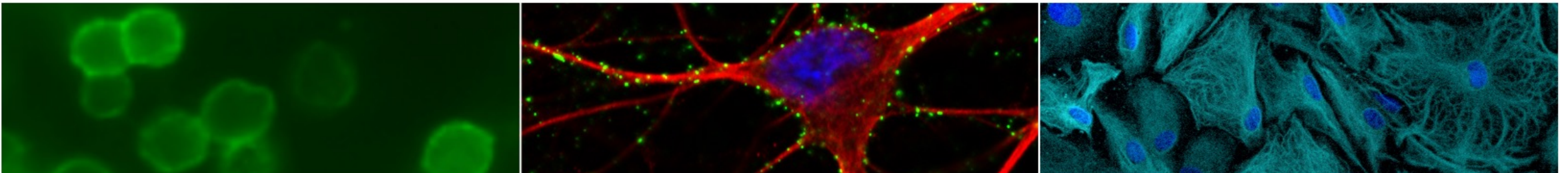
Neuronal cultures



Astrocyte cultures

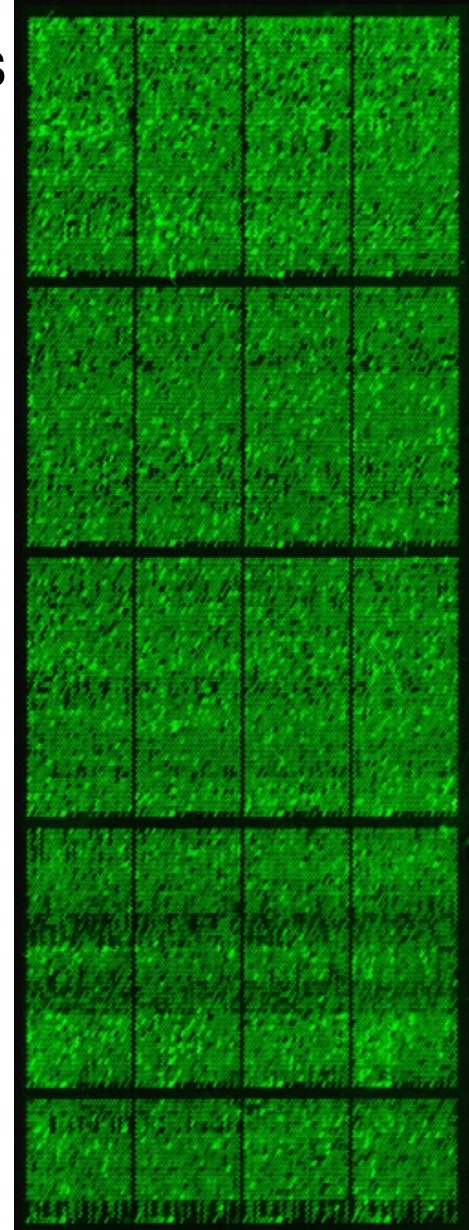
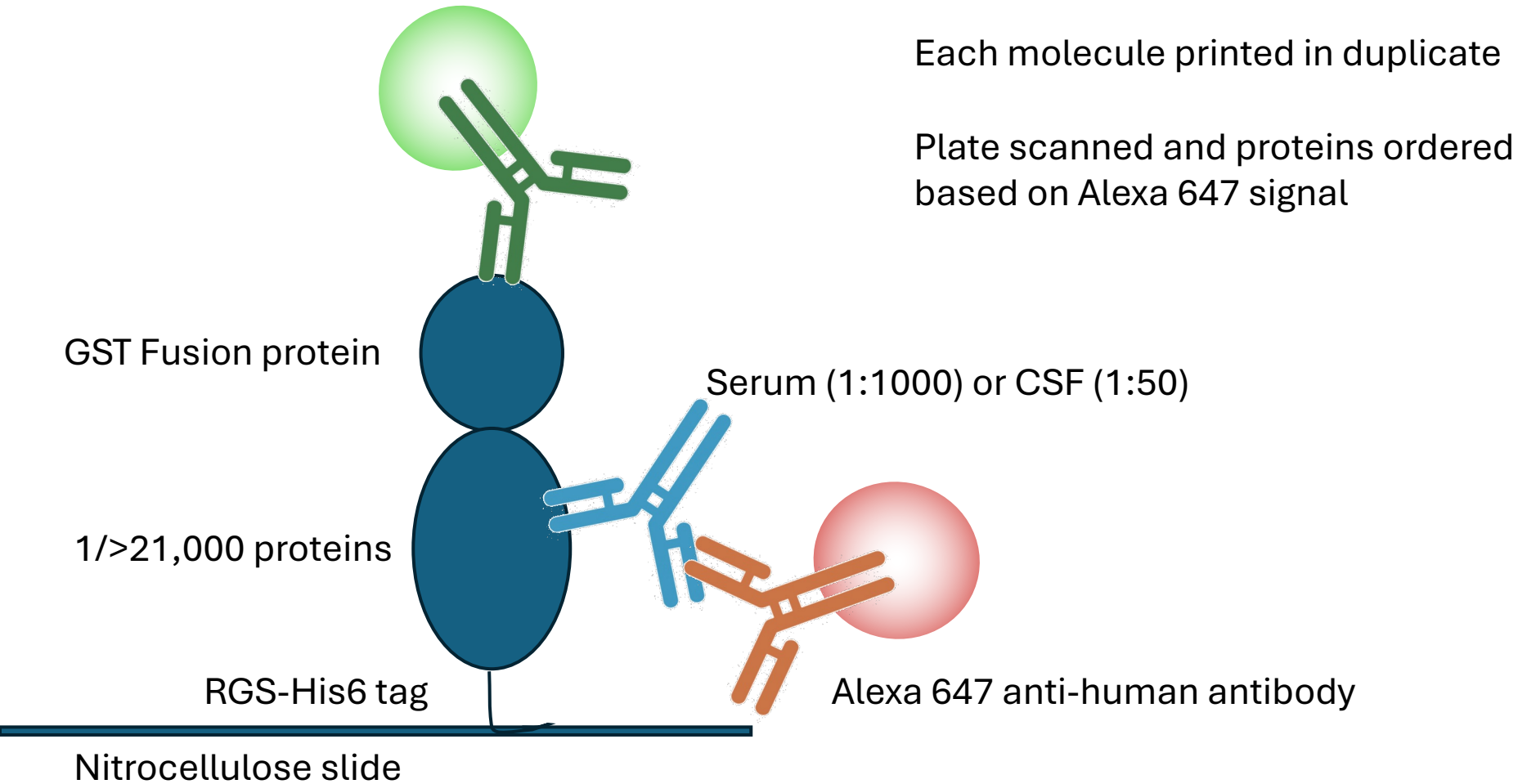
Current needs

1. Identify new targets in people with seronegative disease
2. Global access to affordable diagnostics for known targets
3. Rapid 'bedside' diagnostics
4. Confirmation of a relapse or disease activity in people with an established diagnosis



3D Structure is important for detection of pathogenic antibodies

HuProt v4.0 (CDI Laboratories, Puerto Rico – yeast expressed purified protein array)



3D Structure is important for detection of pathogenic antibodies

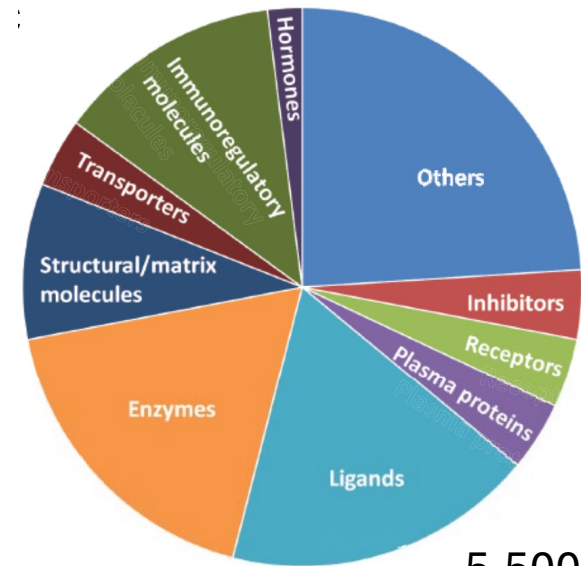
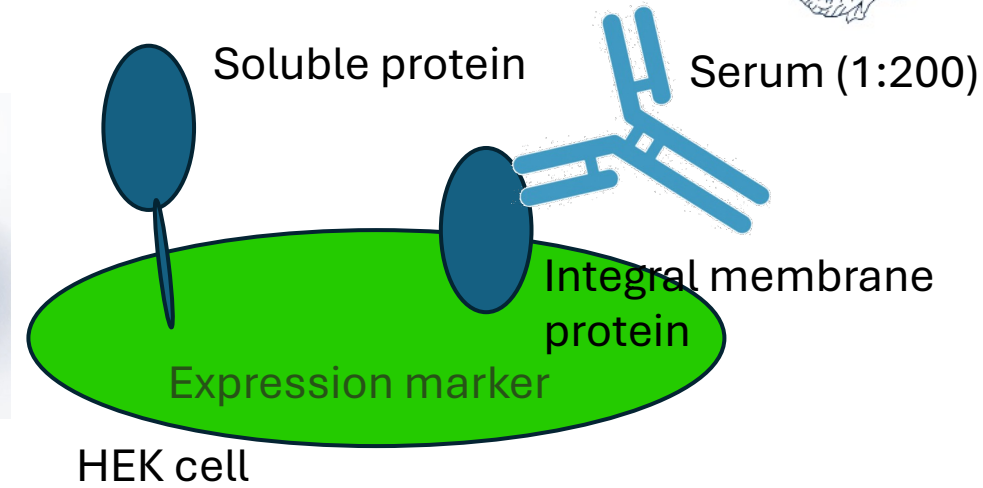
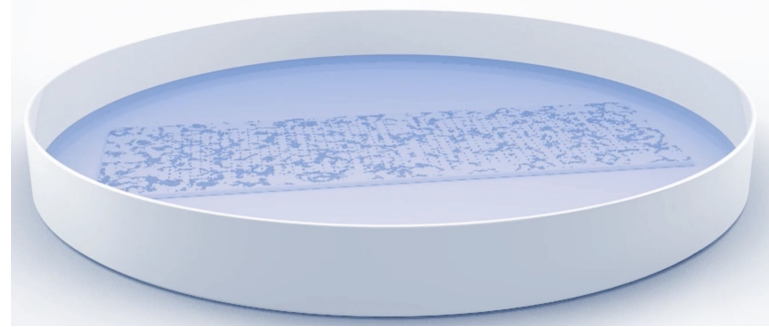
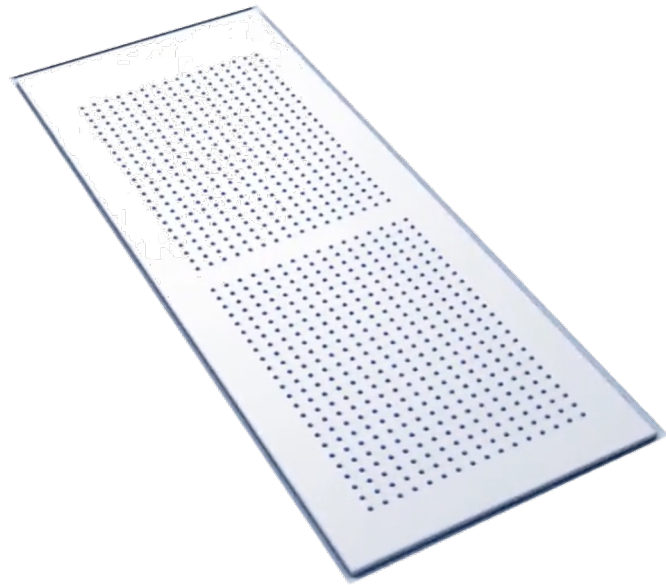
Sub-membrane targets

	Specimen tested	Overall protein rank
Known neural IgG		
AK5	Serum	2
AK5	CSF	26
Amphiphysin	Serum	1
ANNA-1	Serum	39
ANNA-1	CSF	2
ANNA-2	Serum	1
ANNA-3	Serum	2
CRMP5	Serum	3
CRMP5	CSF	3
GAD65	Serum	1
GAD65	CSF	1
GFAP	CSF	24
GRAF	CSF	10
KLHL11	Serum	362
KLHL11	CSF	305
Ma2	Serum	1
NCDN	Serum	1

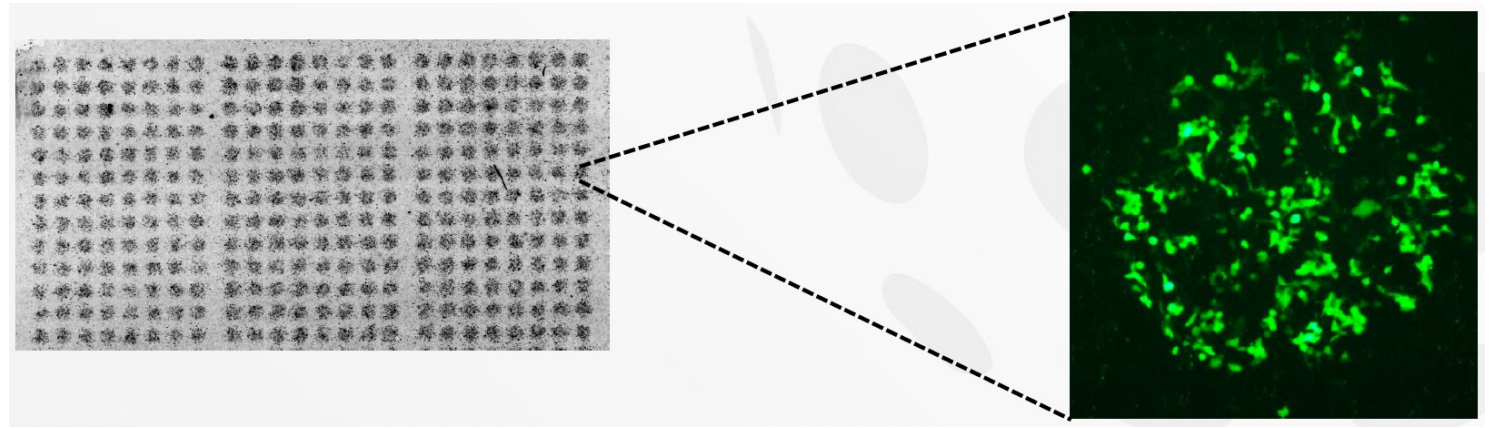
Membrane targets

	Specimen tested	Overall protein rank
Known neural IgG		
AQP4	Serum	12,055
AQP4	CSF	18,010
GABA_BR (Pt 1)	CSF	4896 ^b
GABA_BR (Pt 2)	CSF	Not detected ^b
GRAF	Serum	2324
IgLON5	Serum	17,010
IgLON5	CSF	20,899
LGI1	Serum	12,130
LGI1	CSF	21,661
MOG	Serum	878
NMDA-R	CSF	16,818
Patient IgG		
Neurexin3α (patient 1)	Serum	660

Retrogenix® Cell Microarray Technology (Charles River Laboratories)

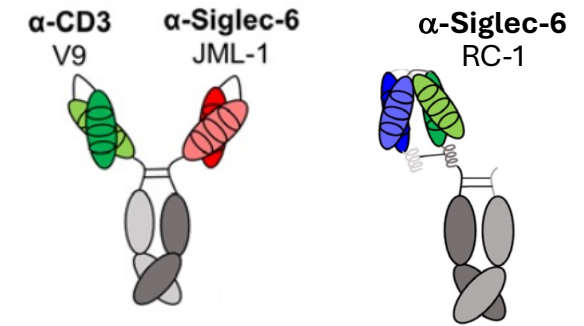
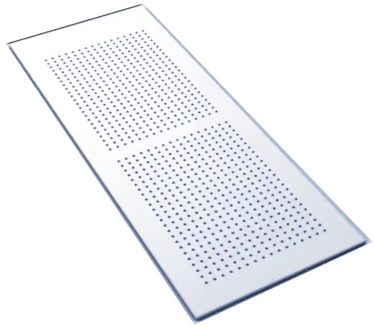


5,500 cDNA in 2020
>6,500 on CR website



Cancer Target¹⁻⁴

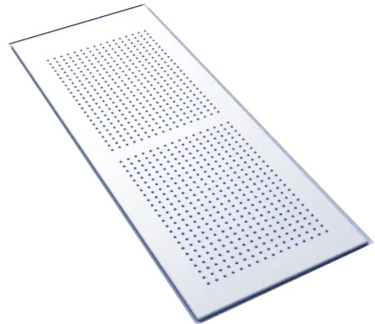
Antibodies produced post alloHSCT a patient with CLL with a good response bound siglec-6



Off-Target effects of mAb⁵

Camrelizumab an anti-PD1 mAb - >80% treated patients developed capillary hemangioma

Camrelizumab also bound and stimulated VEGF2 Receptor which leads to capillary blood vessel proliferation



1. Chang et al. Cancer Immunology Research 2018
2. Cyr et al. J. Immunother Cancer 2022
3. Kovalovsky D et al Leukemia 2021
4. Jetani et al Blood 2021
5. Finlay et al mAbs 2018

Global access to affordable diagnostics against known targets

Rapid diagnostics

Diagnostic Fellowships



Sri Lanka



Mr. Wasantha Pushpakumara



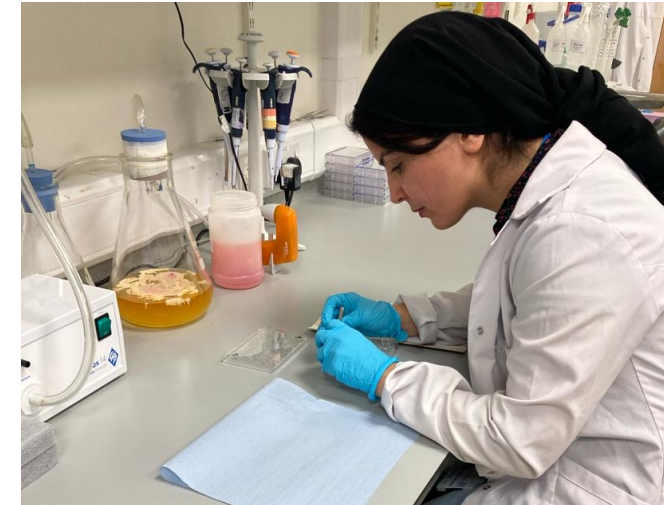
Dr. Danushka Dasanayake

Zambia



Ms. Caroline Kabwe

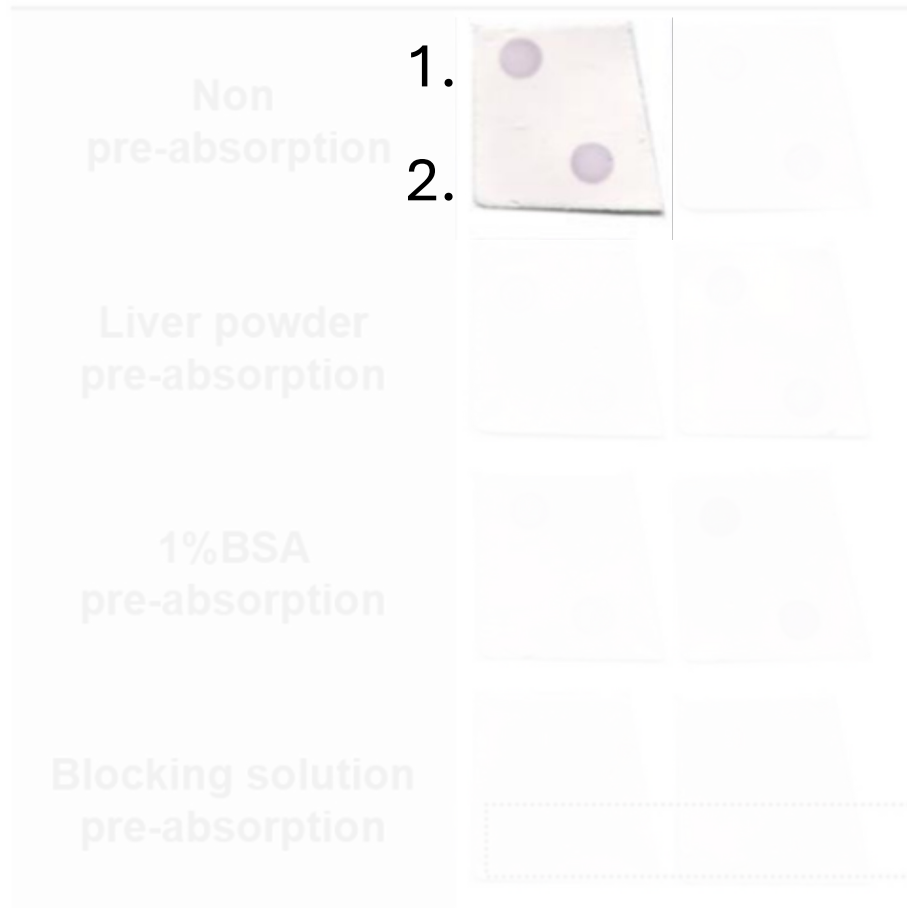
Iran



Dr. Zhila Maghbooli





Rapid idot

1. M23-AQP4 transduced HEK cell extracted arrays (differential centrifugation and native HPLC)
2. Control HEK cell extract
3. 25 ul serum diluted 1:10 with PBS
4. Sheep anti-human IgG – AP (alkaline phosphatase)
5. Substrate: BCIP/NBT











Rapid idot method – Block is key to success

1. M23-AQP4 transduced HEK cells extract
2. Control HEK cell extract

Preconditioning of serum	AQP4-IgG Negative	
None		
Liver powder pre-absorption		
1%BSA pre-absorption		
Blocking solution pre-absorption		








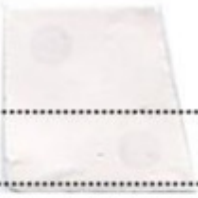
Rapid idot method – Block is key to success

1. M23-AQP4 transduced HEK cells extracted arrays (differential centrifugation and native HPLC)
2. Control HEK cell extract

Preconditioning of serum	AQP4-IgG Negative	
None		
Liver powder pre-absorption		
1%BSA pre-absorption		
Blocking solution pre-absorption		











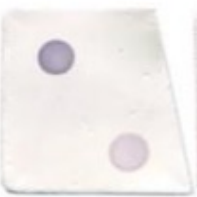













Rapid idot method – Block is key to success

1. M23-AQP4 transduced HEK cells extract
2. Control HEK cell extract

Preconditioning of serum	AQP4-IgG Negative	
None		
Liver powder pre-absorption		
1%BSA pre-absorption		
Blocking solution pre-absorption		

Rapid idot method – Block is key to success

1. M23-AQP4 transduced HEK cells extract
2. Control HEK cell extract

Preconditioning of serum	AQP4-IgG Negative		Low titer		High titer		Nonspecific binding
None							High
Liver powder pre-absorption							High
1%BSA pre-absorption							High
Blocking solution pre-absorption							Low

Rapid idot method – Substrate is stable over 1 year

eTable 1. Stability evaluation of IDoT assay			
	Conditions	n	Percent agreement
Within-lot			100
	1 month	40	
	2 months	40	
	3 months	40	
	12 months	40	

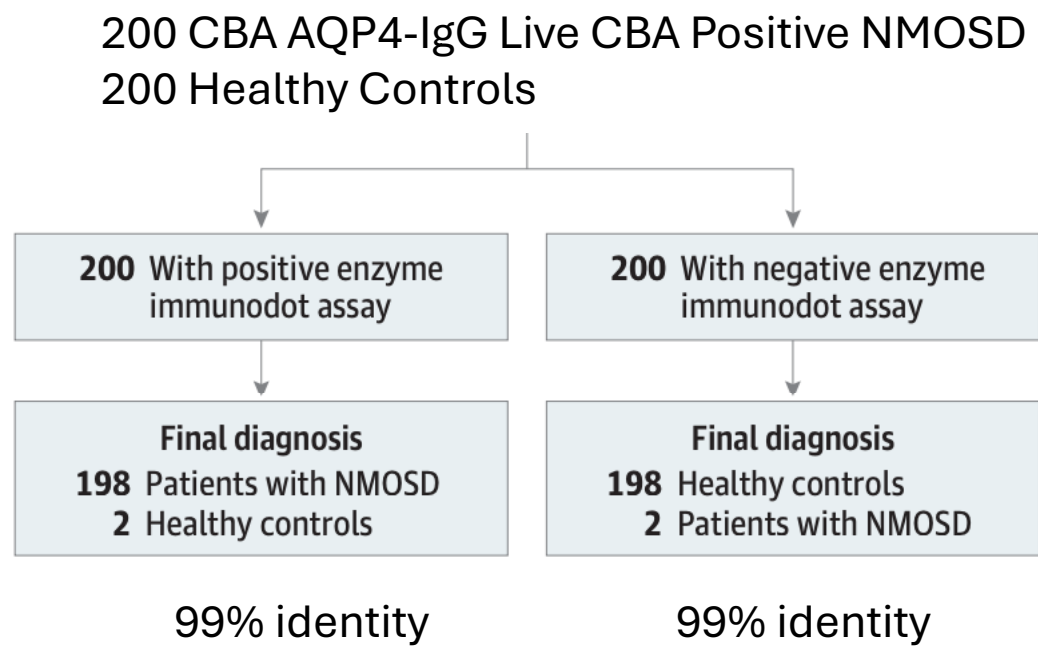
Rapid idot method – Data

Prospective multicentre study

Patients The Third Affiliated Hospital of Sun yat-sen University, Guangzhou (China)

Patients First Affiliated Hospital of Fujian Medical University, Fuzhou (China)

Live M23 CBA Shaanxi Normal University (China)



Other diseases

0/116 MS

0/50 SLE

0/34 SS

0/49 MG

1/22 HAM

0/4 Others

1/275 Total

Immunodot assay results	International cross-validation in Korea	
	Positive	Negative
Positive	47	1
Negative	0	25
Total	47	26

Prof. Ho Jin Kim

Looking for £20K funding support for this project

1. AQP4-IgG positive. 2. MOG-IgG clear positive. 3. MS - Clinically definite. 4 Any difficult samples for any platform



Confirmation of a relapse or

Evidence of disease activity in people with an established diagnosis

iPCR

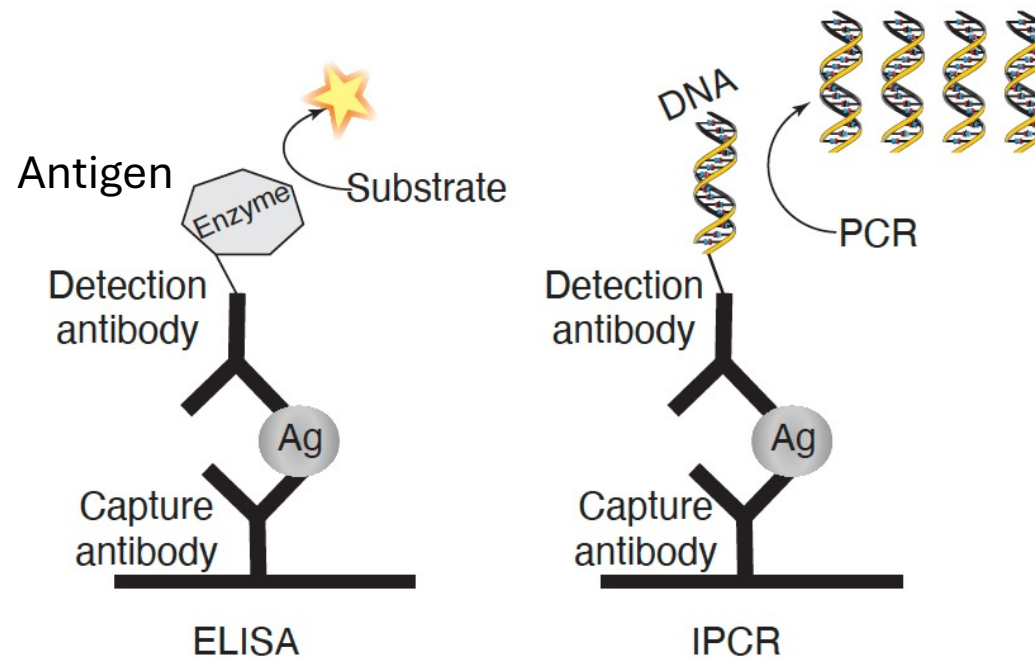
molecules of antigen needed for detection

ELISA: 58,000,000

(5.8×10^6)

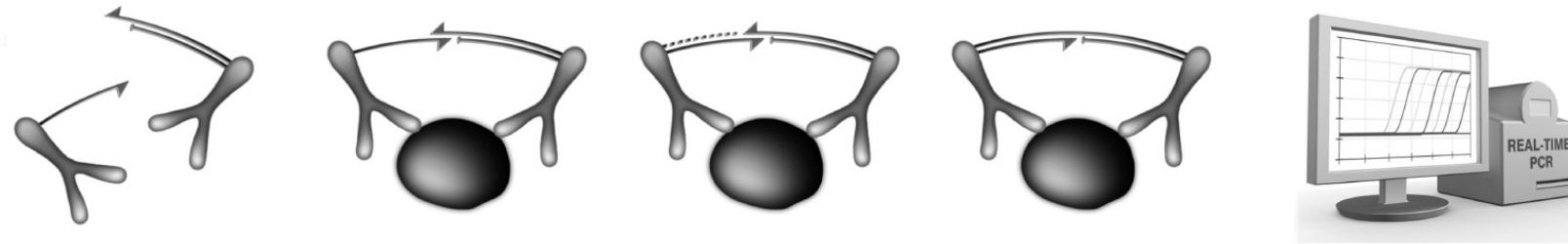
iPCR: 580

(5.8×10^2)



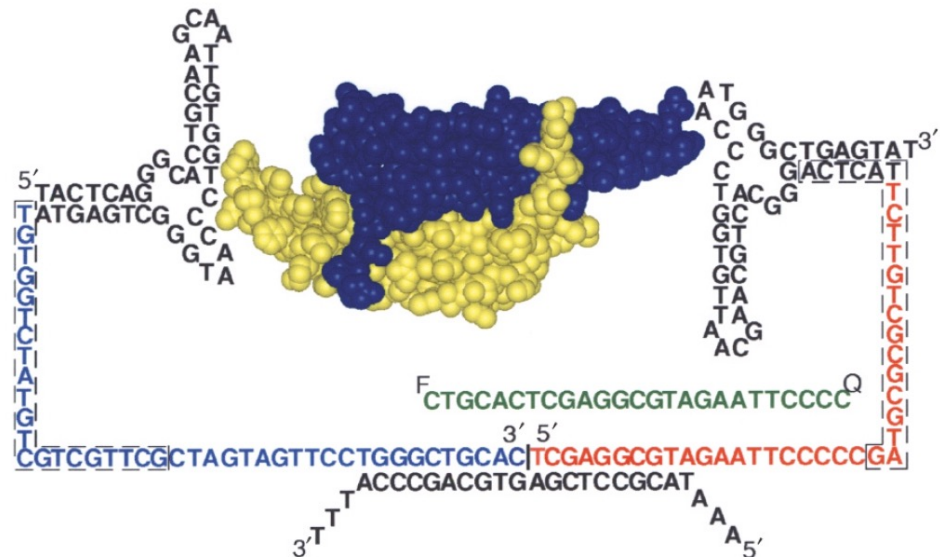
iPCR (depending on the molecule) is 5x to 1,000,000,000x more sensitive than ELISA in published head-to-head comparisons.

Proximity extension assays



Antibody based

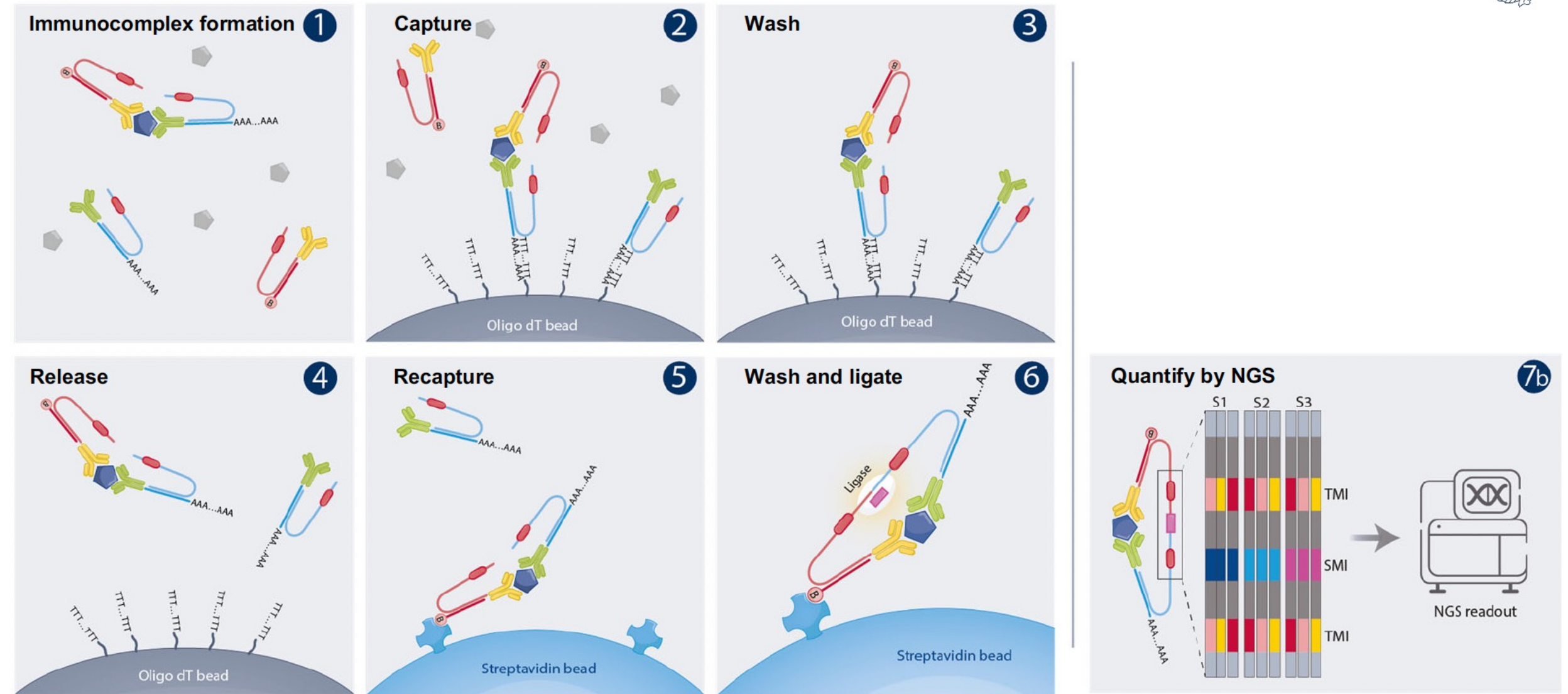
Proximity dependent DNA-ligation assays



Aptamer based

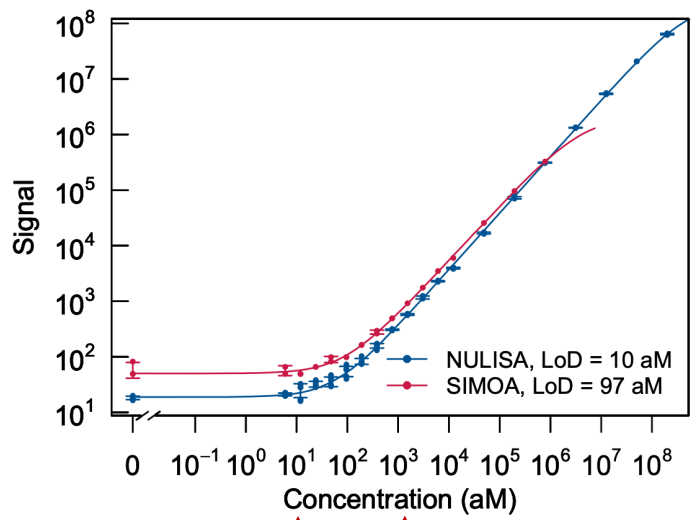
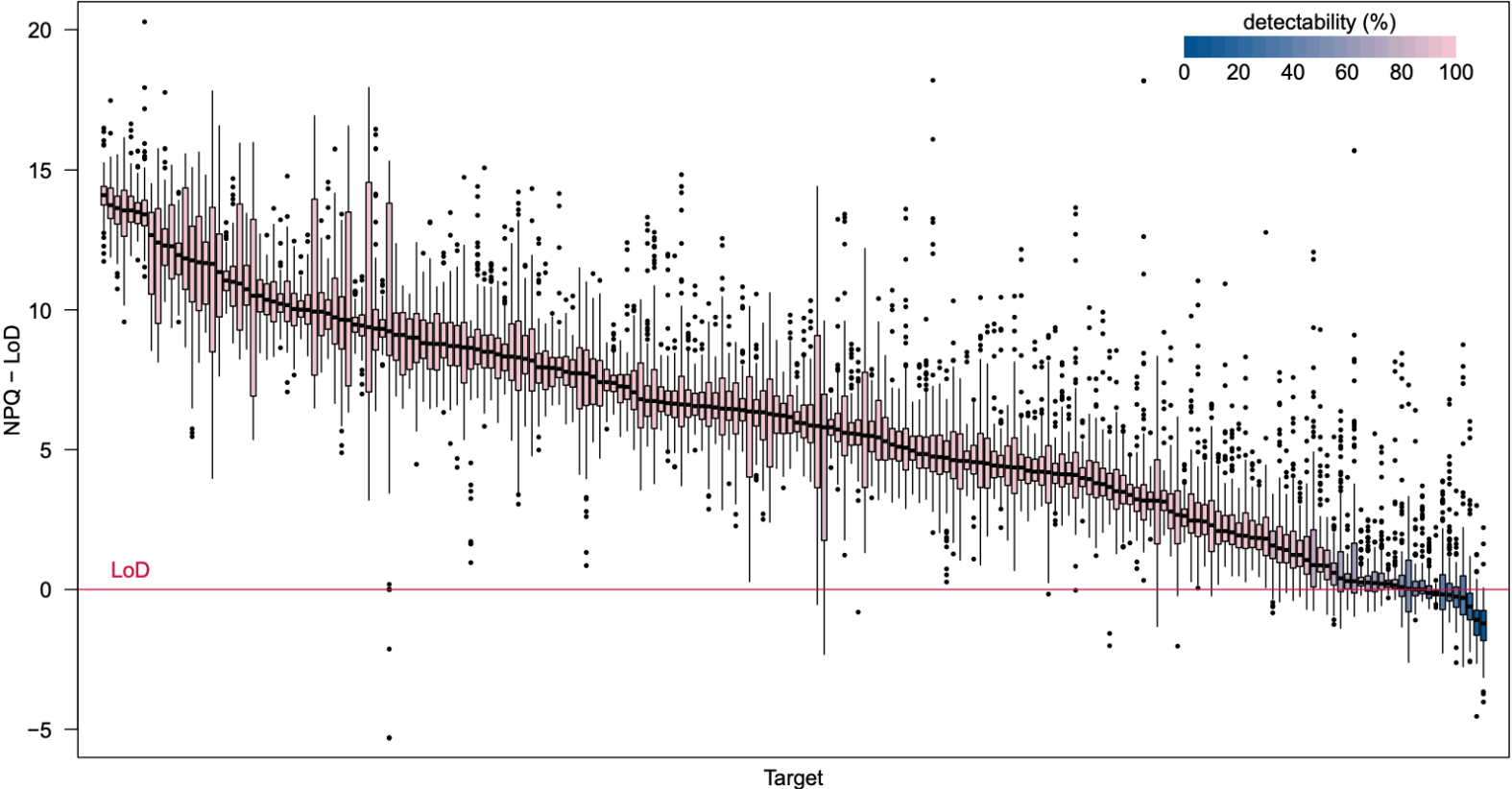
NULISA – Nucleic acid Linked Immuno-Sandwich Assay

All good for soluble molecules or with domains that are not affected by extraction from their native environment



p24

NULISA

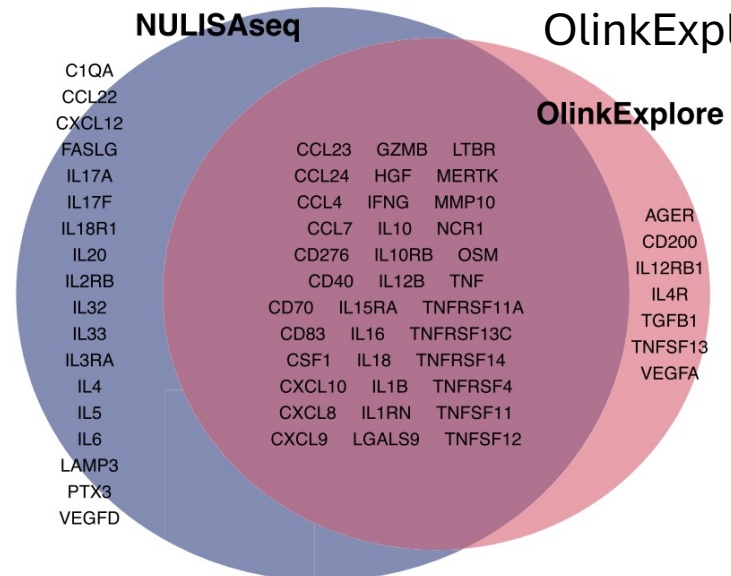


10 1000
molecules

151 samples, 204 targets

NULISAseq

OlinkExplore



Summary



- Global access to affordable antibody testing is needed
- Rapid idot or alternative point of care testing may be possible
- Retrogenix cell microarray looks useful, if multisubunit channels are added to their repertoire it may be even more useful
- iPCR based techniques are at the Attomolar detection level
i.e. 10-1000 molecules. Only limitation now is pairs of high affinity antibodies or aptamers.

AQP4 specific mAb 50,000,000,000 (50 Billion ~12 ng)
to 600,000,000 (600 million ~150 pg)

