EDCTP Contract N^0 RIA2020EF-2918

Rapid diagnostics for COVID-19: manufacturable in Africa to increase affordability, improve epidemic preparedness and strengthen local resilience

Project Information

Grant Code	RIA2020EF-2918
Project Full title	COVID-19 Diagnostics for Africa
Project Acronym	AfriDx
Funding Scheme	EDCTP
Start Date of the Project	1 October 2020
Duration	15 Months
Project Coordinator	(UCAM)
Project Website	https://afridx.ceb.cam.ac.uk/

Deliverable Information

Deliverable Number	5.1
Deliverable Title	Modified and characterised high-affinity scFv
Workpackage Number	WP5
WP Leader	UCAM
Authors	Samir Hamaia (UCAM)
Contributors	UCAM
Reviewers	Lisa Hall (UCAM)
Contractual Deadline	30 April 2021
Actual Delivery Date	22 Feb 2021

History of changes:

Date	Version no	Comments
22 Feb 2021	1.0	
30 Aug 2021	1.1	Updated results

Delivery Type

R	Report	

DEM	Demonstrator, pilot, prototype, plan designs, new or revised health policies etc	✓
DEC	Websites, patents filing, press & media actions, etc	
OTHER	Other	

Dissemination Level

PU	Public	✓
RE	Restricted to a group specified by the consortium.	

List of content

Partner	Contribution to this deliverable
S. W Hamaia	Has carried out all the work described below in collaboration with A.P. Jackson and E A H Hall

1 Status of the Deliverable

(Describe in a few sentences the status of the Deliverable: Final or partly completed. If the Deliverable is not final, what is the new time schedule? What are the future plans in connection with this deliverable).

SWH screened an existing single chain fragment antibody (scFv) library for high-affinity and highly specific His-tagged scFvs against human IgM and human immunoglobulins. High affinity and highly selective scFvs were identified by ELISA binding. This deliverable was successful.

2 Summary of the results (max. 1-2 pages)

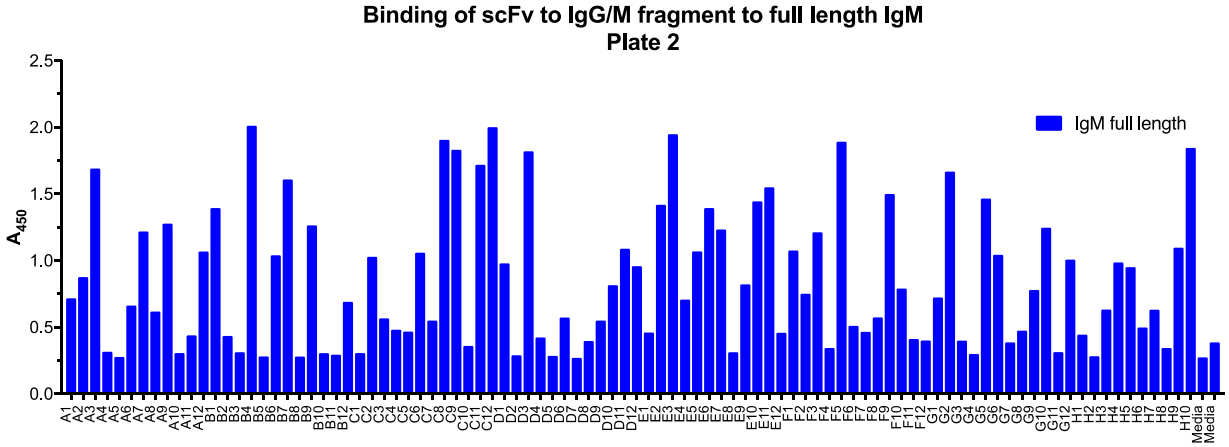
(Make a short summary the work progress. Also summarize the work of contributing partners.)

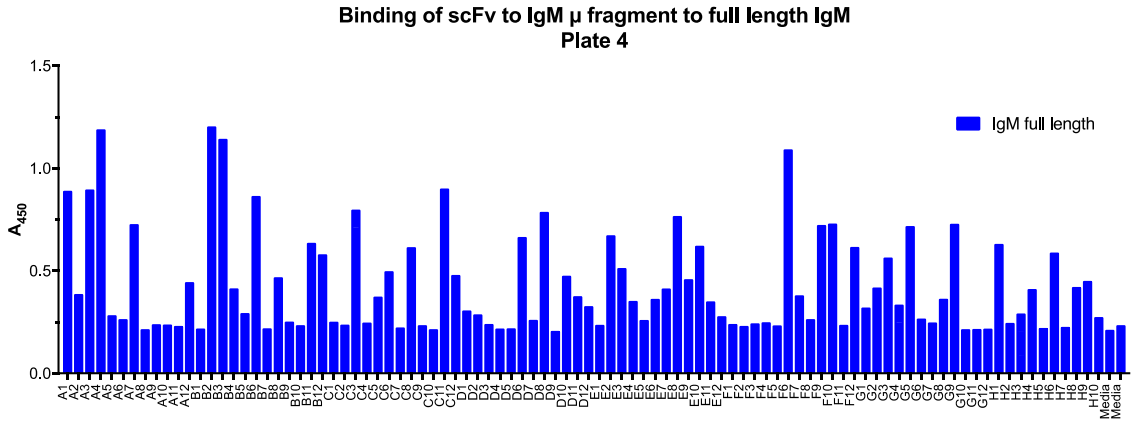
The scFv library was screened against human IgM and human IgG. Positive clones identified by standard ELISA techniques. Highest binders have been tested for specificity and affinity. cDNAs for these scFvs have been sequenced and cloned into expression vectors for use in Africa

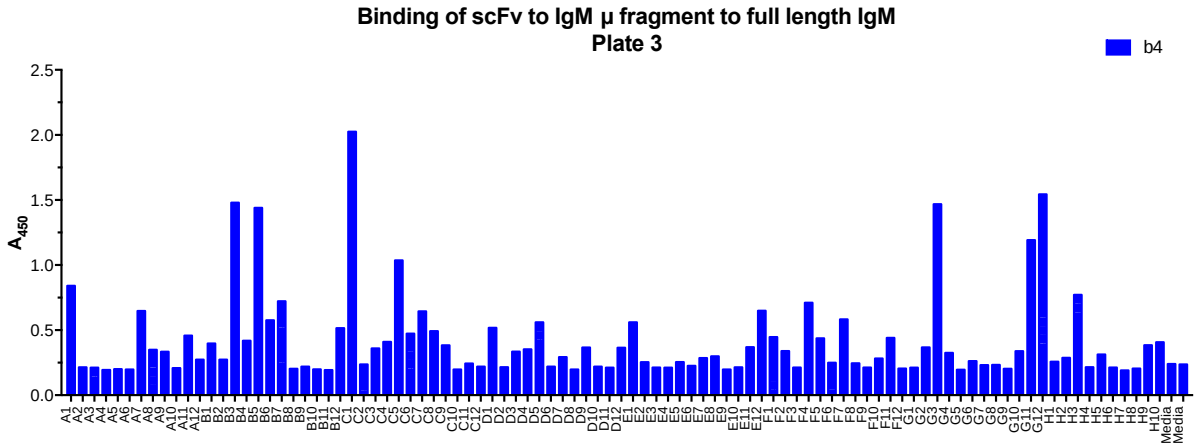
3 Description of work performed and obtained results

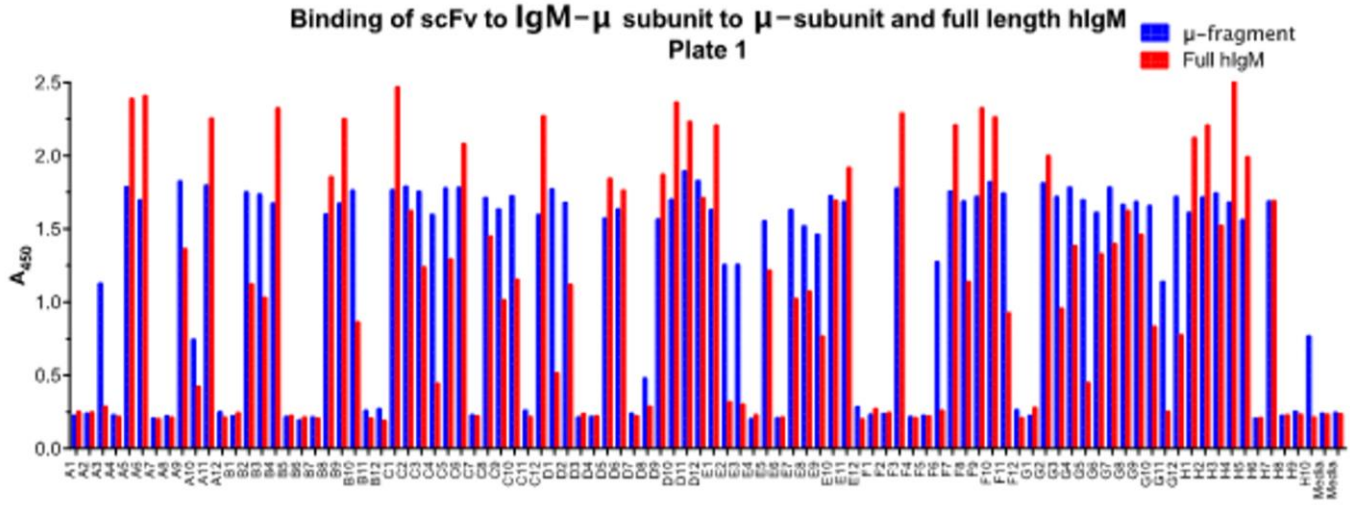
(Describe the work performed and obtained results towards the deliverable).

Please include tables and figures if possible. All data should be included
Screening ELISAs showing results of whole library screens for scFvs against human IgM (full length and defined fragments)

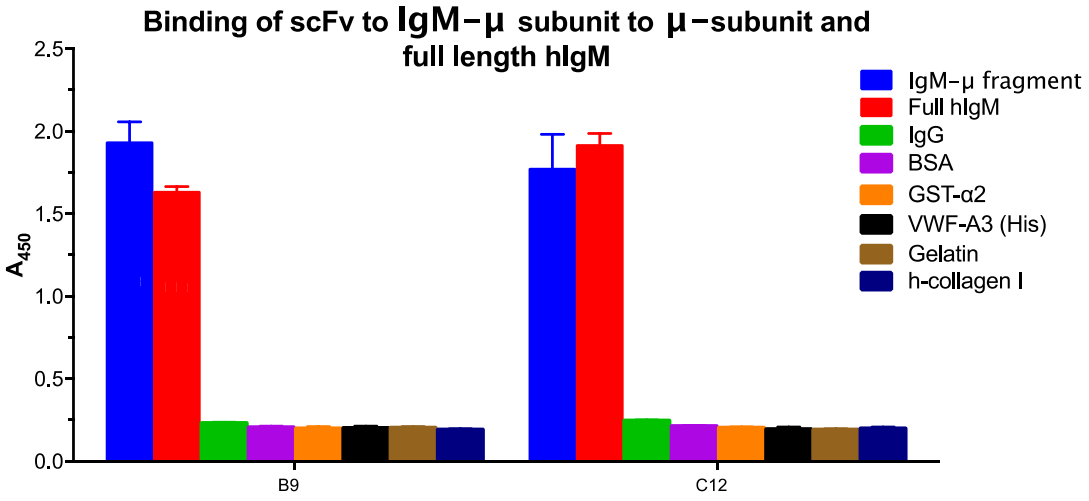


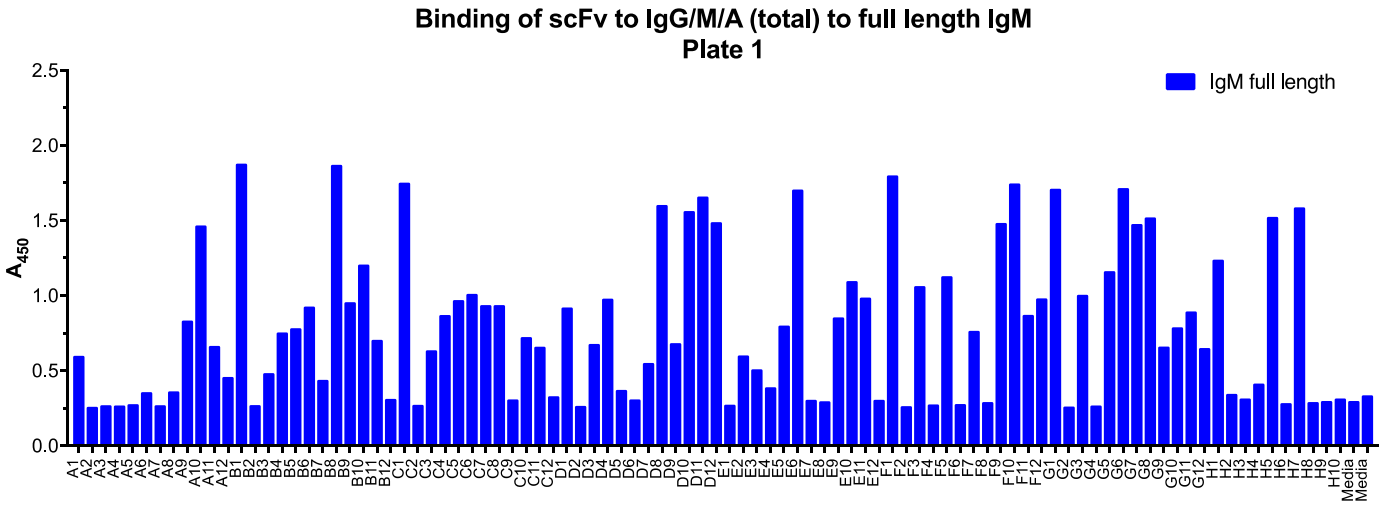




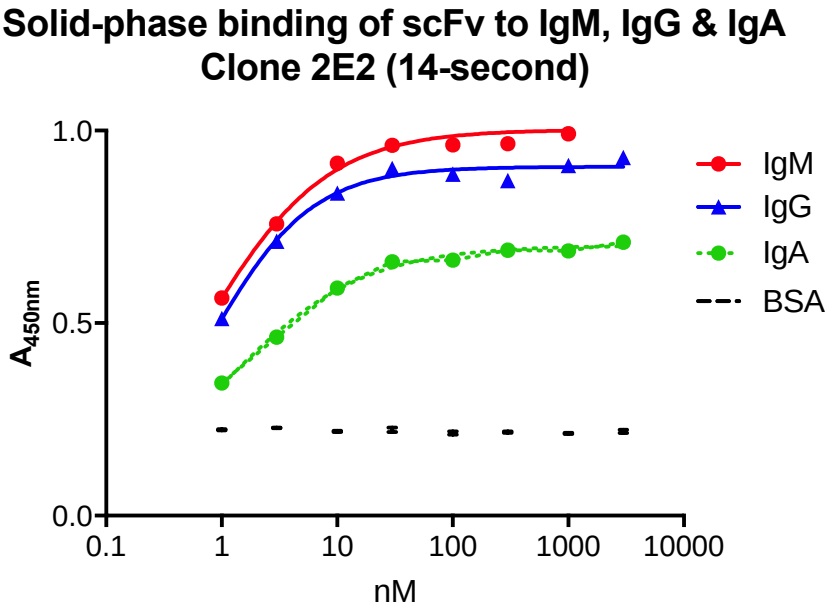


High selectivity of scFvs against human IgM

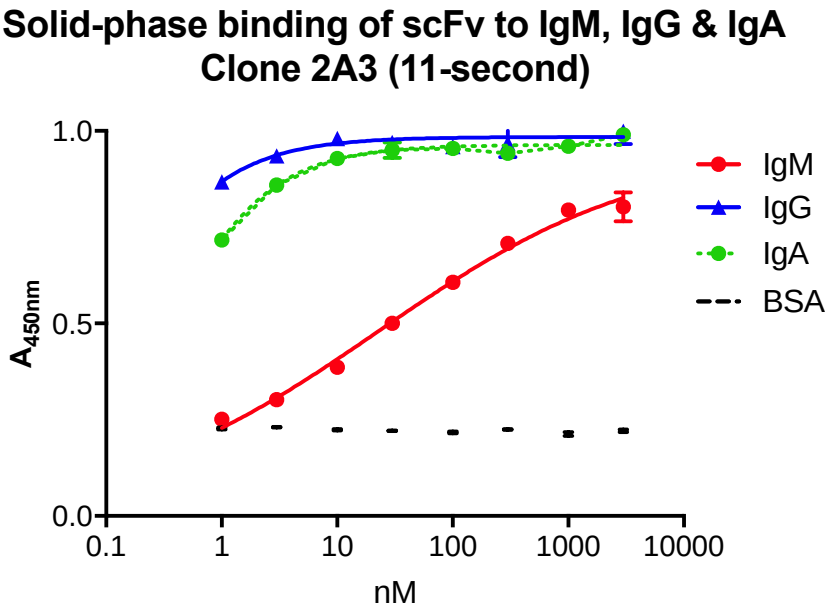




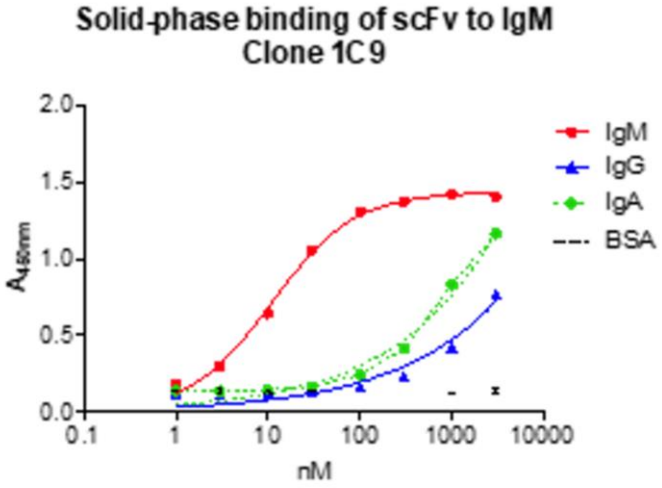
Quantitative selectivity assays for selected high affinity scFvs



	IgM	IgG	IgA
Kd	0.7355	0.7735	1.094

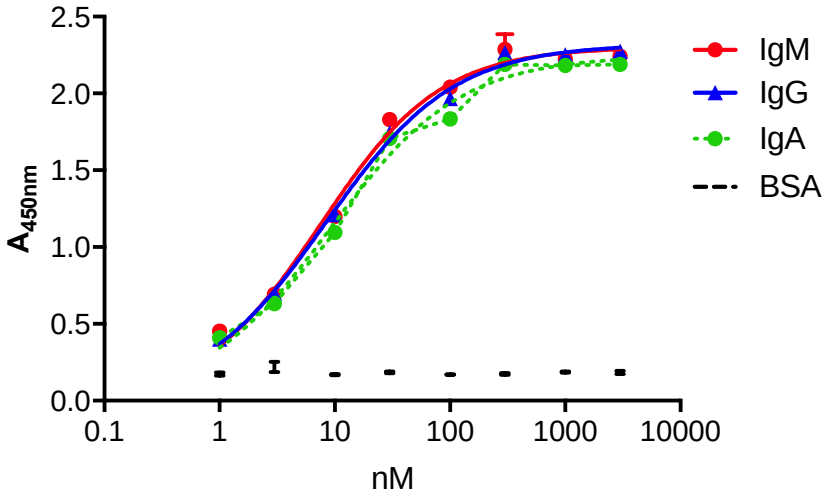


	IgM	IgG	IgA
Kd	22.84	0.1031	0.3186



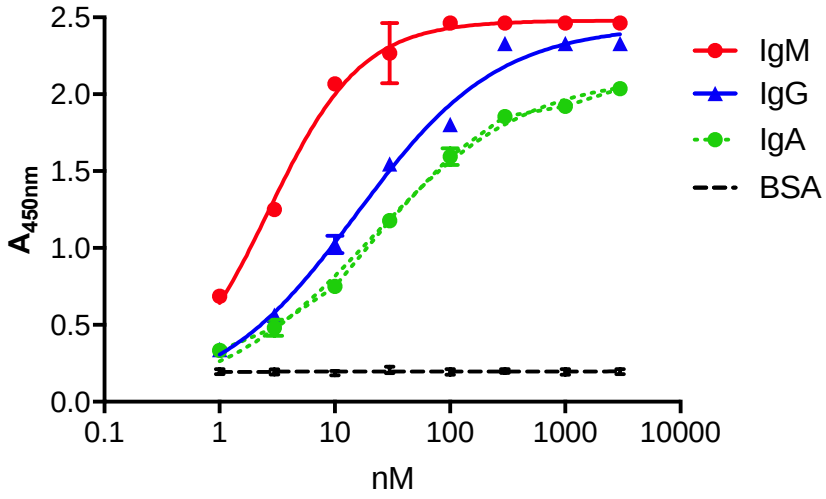
	IgM	IgG	IgA
Kd	11.12	~ 1424488786075	~ 133680966072

Solid-phase binding of scFv to IgM, IgG & IgA
Clone 3D2

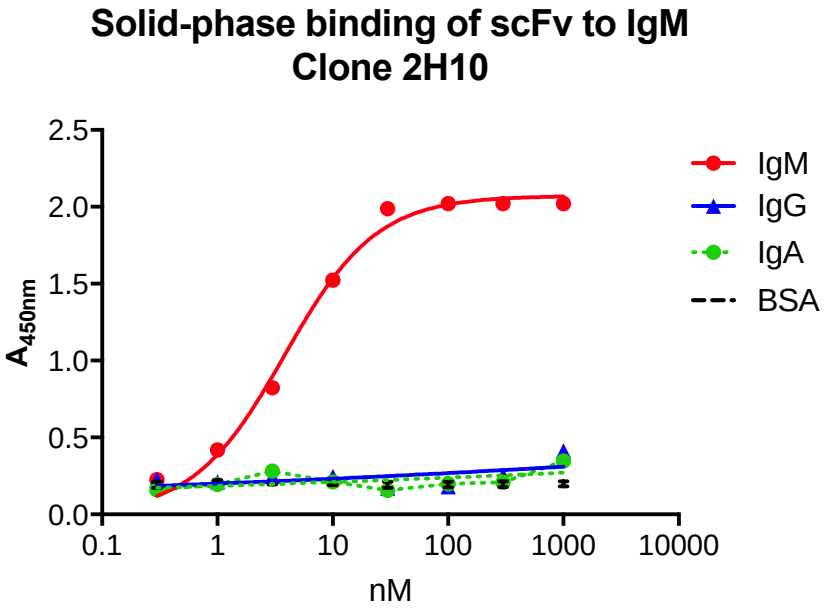


	IgM	IgG	IgA
Kd	7.579	8.388	9.208

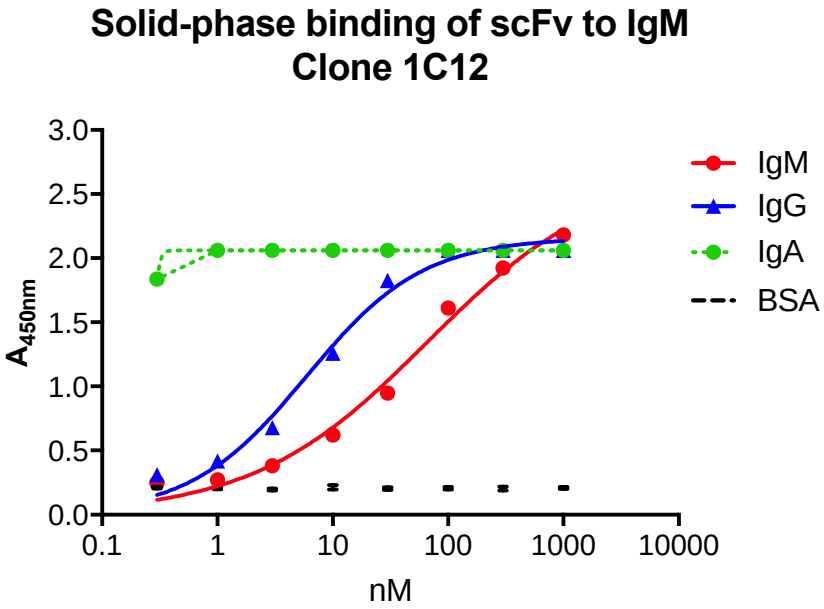
Solid-phase binding of scFv to IgM, IgG & IgA
Clone 1D11



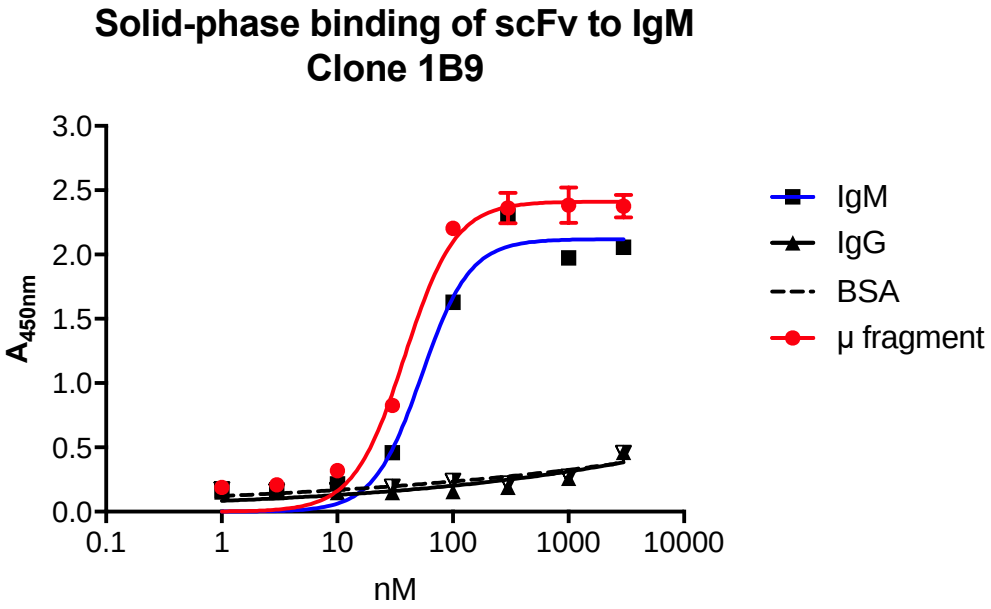
	IgM	IgG	IgA	BSA
Kd	2.658	15.51	20.65	6.853e-019



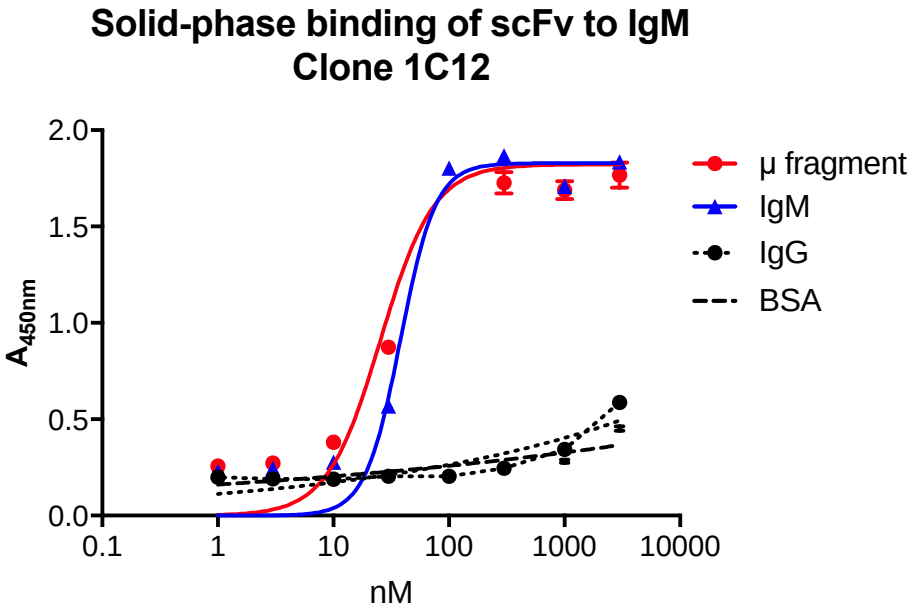
	IgM	IgG	IgA
Kd	3.831	8.396e+031	1.74e+029



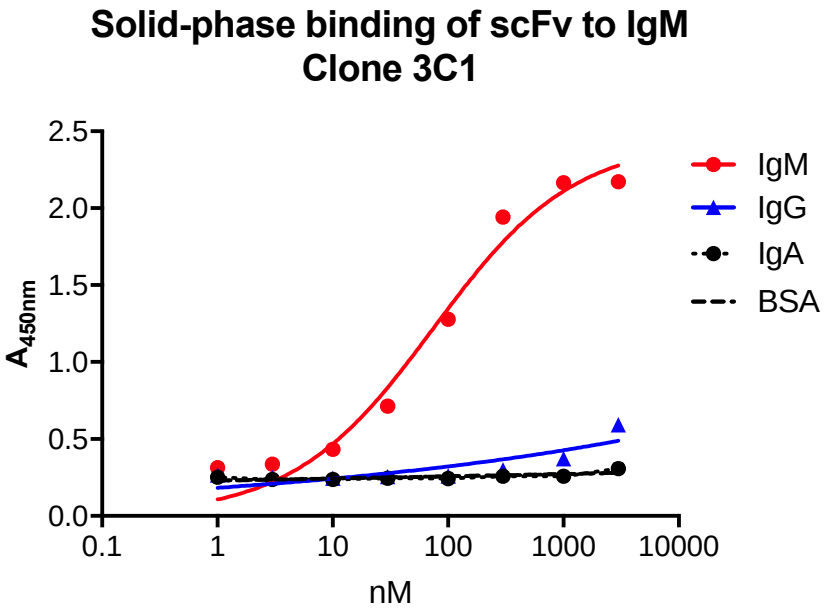
	IgM	IgG	IgA
Kd	65.81	5.943	~ 0.2649



	µ fragment	IgM	IgG	BSA
Kd	38.21	53.66	~ 9.528e+019	~ 2.433e+023

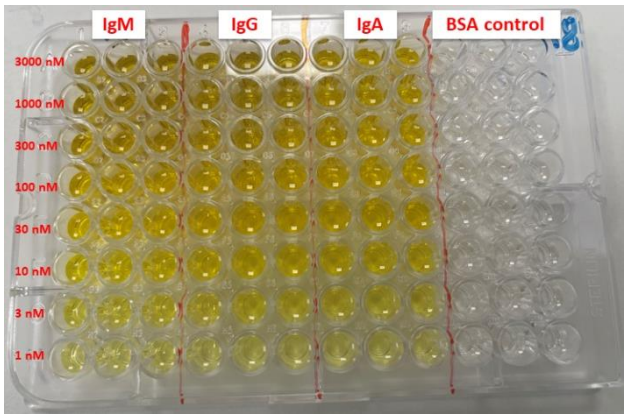


	µ fragment	IgM	IgG	BSA
Kd	25.55	38.1	~ 4.391e+019	2.735e+029

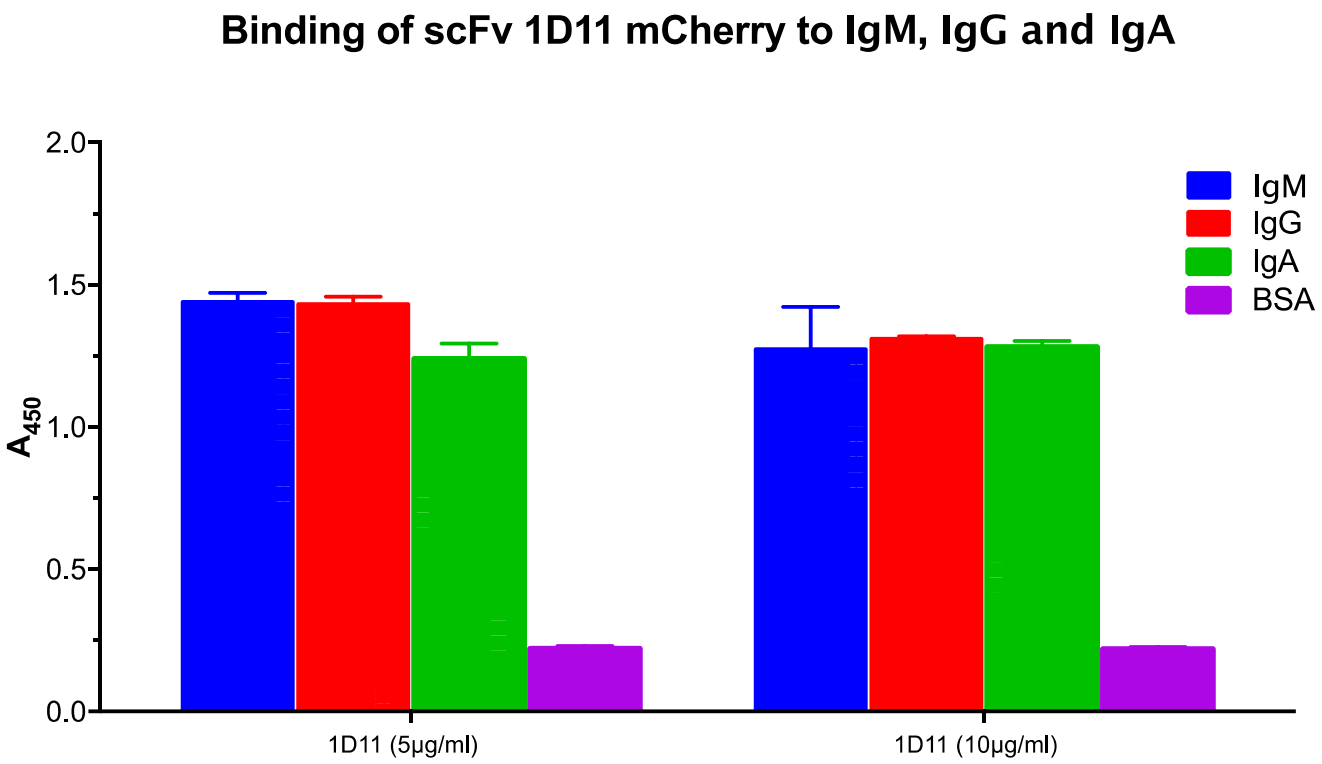


	IgM	IgG	IgA	BSA
Kd	74.69	5.365e+027	7.865e+030	5.281e+033

For selected high affinity and high selective scFvs, experiments were performed to incorporate suitable fluorescent tags (eg MCherry). We find that about 70% of such scFvs can successfully incorporate MCherry fluorescent tag . These were then screened for binding to human Ig targets and those that retain their binding activity were identified. These will be candidates for further assay development.



For scFvs incorporating mCherry, the selectivity of the scFv/mCherry construct does not change



Conclusions:

scFvs selective for human immunoglobulin classes can be obtained using standard screening procedures for scFvs. Selected scFvs can be successfully modified by incorporation of mCherry fluorescent protein tag into the scFvs, with no compromise on selectivity.