R SHINY WORKSHOP

Introduction to Luminex

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xMAP: Multiplexing Technology

xMAP technology allows users to perform a wide range of protein-and nucleic acid-based multiplex assays which can simultaneously detect up to 500 targets in a single run

Advantages of xMAP technology

- Test for more biomarkers

- Develop custom assays

- Use less sample

- Save time and reagents

- Gain a better understanding of complex biological systems

What is multiplexing? Multiplexing is a method for high-volume biomarker testing – or testing multiple analytes simultaneously within a single run using a single sample volume. xMAP technology is best-suited for testing 3-500 targets

xMAP: Multiplexing Technology

How does xMAP technology work? xMAP technology uses labeled microspheres or beads, allowing for the simultaneous capture of multiple analytes from a single reaction. Because of their small size and low-density microspheres-based assay exhibit virtual solution phase kinetics during the reaction i.e., the beads are individually read using an xMAP instrument

What does xMAP mean? Multi-Analyte Profiling, where the 'x' represents the biomarkers (such as proteins, nucleic acids, or polysaccharides) that are being tested

xMAP Beads come in a variety of formats, including magnetic and non-magnetic. xMAP beads pass through a red laser, or LED, which excites the internal dyes to distinguish the microsphere set. Then, a green laser or LED excites the fluorescent reporter dye to determine the result of the assay

xMAP Technology: The Science

Magpix is the simplest and most affordable of Luminex's xMAP instruments

Compact multiplexing unit performs up to 50 different tests in a single reaction volume and reads a 96 well plate in just 60minutes

Key Features:

Multiplexing up to 50 analytes per sample

Sensitivity: approximately 10^6 copies of DNA or single-digit picogram levels of protein

Selection: flexibility of panels and formats

Read time: 96 well plate <60 minutes (up to 4,800 tests/hour)

Daily start-up/Shut-Down: <15minutes

Footprint: 3 ft linear bench space


MAGPIX®

The assay
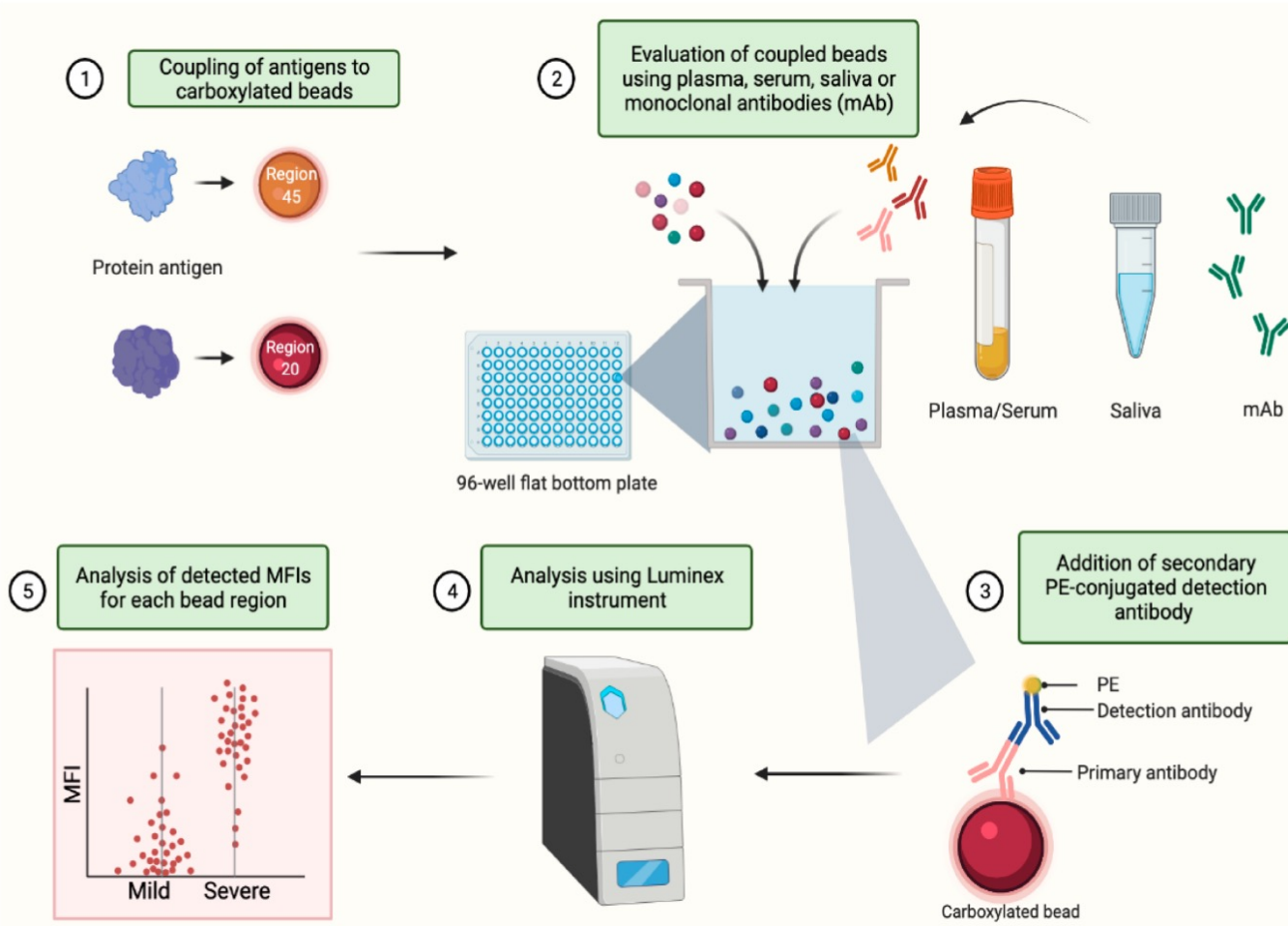
Luminex protocol involves several steps:

1. Coupling of antigens to carboxylated beads

2. Evaluation of coupled beads using plasma, serum, saliva or monoclonal antibodies

3. Addition of PE-conjugated secondary antibody (detector antibody)

4. Analysis on Luminex instrument

5. Analysis of detected MFIs for each bead region

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The assay – bead coupling

Optimal protein amount to couple to magnetic beads is first determined
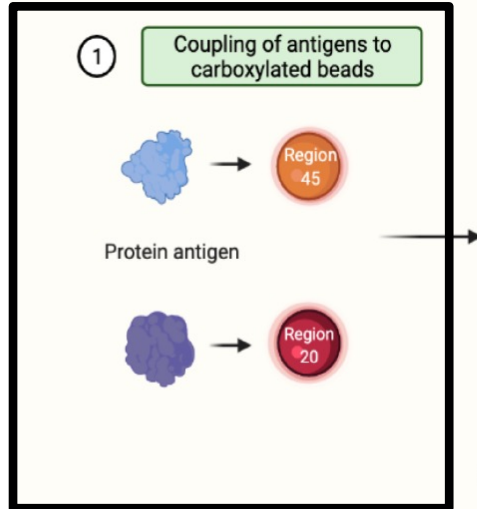
All proteins initially coupled 6.25 x 10^5 pre-activated beads at various protein concentrations (trial coupling)

The optimum antigen concentration is then determined by observing what concentration of protein resulted in a log-linear standard curve using the convalescence plasma

This is important in achieving an optimal dynamic range to be able to measure a wide range of different concentrations whilst measuring all samples at the same dilution

Once determined, we perform a bulk coupling 2.5 x 10^6 pre-activated beads (can be used for 3000 samples)

The assay – bead coupling

a

IgG SARS-CoV-2 antigens

MFI / standard curve dilution

antigen
- NP
- RBD
- S1
- S2
- Spike
- Main protease
- Papain-like protease

b

IgG non-SARS-CoV-2 antigens

MFI / standard curve dilution

antigen
- 229E S1
- fluA H1N1
- fluA H3N2
- fluB Phuket
- fluB Victoria
- HKU1 Spike
- NL63 NP
- OC43 Spike
- tetanus
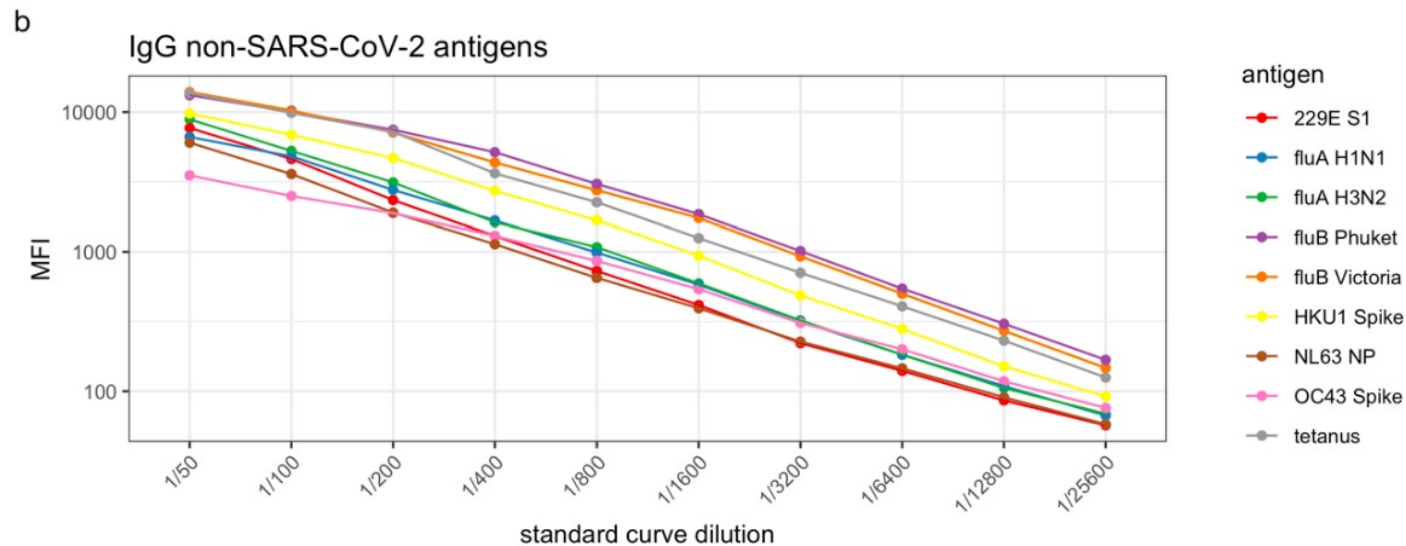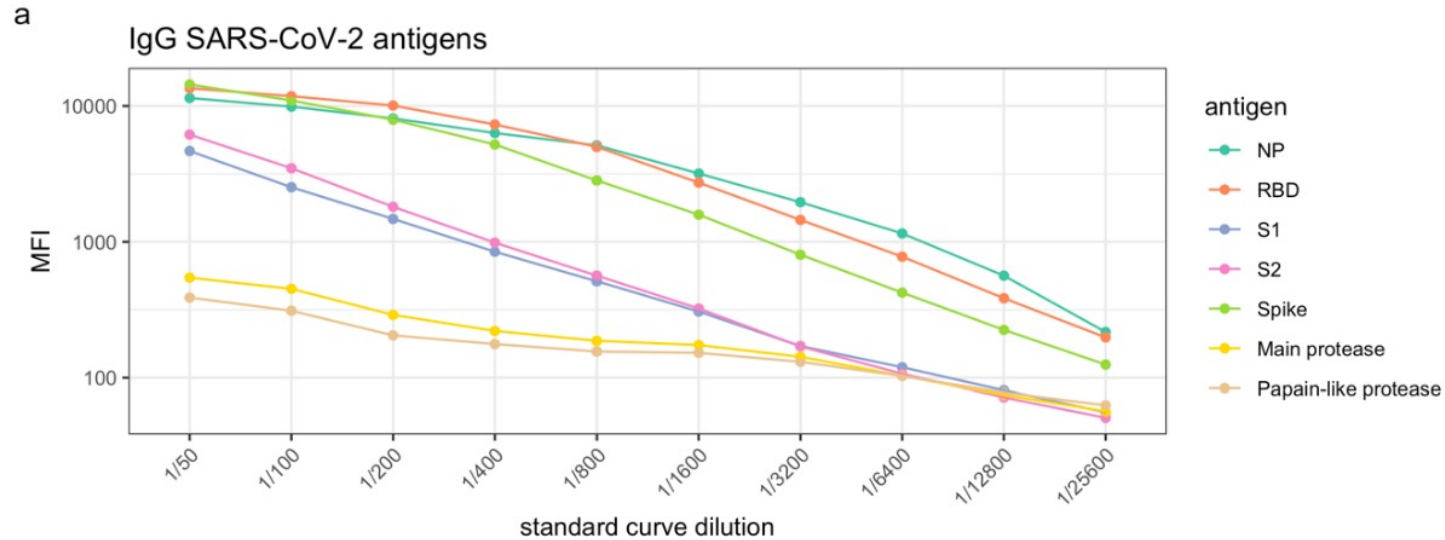
Standard curves for SARS-CoV-2 antigens and other antigens in human plasma, such as respiratory viruses and human seasonal coronaviruses

Levels of IgG antibodies are presented as median fluorescence intensity (MFI) values

Plasma used consists of convalescent plasma from 10 SARS-CoV-2 qPCR positive individuals collected approximately 30 days after onset of symptoms

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Covid Panel

Pathogen	Antigen region	Description
SARS-CoV-2	S1	SARS-CoV-2-Spike Glycoprotein (S1), recombinant antigen AA 1–674
SARS-CoV-2	S2	SARS-CoV-2-Spike Glycoprotein (S2) recombinant antigen AA 685-1211
SARS-CoV-2	NP	SARS-CoV-2 Nucleocapsid protein, full length AA 1-419
SARS-CoV-2	RBD	SARS-CoV-2-Receptor Binding Domain (RBD) of S1
SARS-CoV-2	Spike (trimer)	SARS-CoV-2 Full length Spike trimer
HCoV-NL63	NP	Recombinant c-terminal NP, contains 130 AA
HCoV-229E	S1	Recombinant S1 subunit, AA 19-717
HCoV-HKU1	Spike	In house from collaborator
HCoV-OC43	S1+S2+ECD	Recombinant Spike protein (S1 + S2 + ECD), AA 1-1304
IVB Vic 02/1987	Hemagglutinin	Influenza B HA1, AA 1-362
Tetanus Toxoid		Clostridium tetani (formaldehyde inactivation)

The assay – plate setup
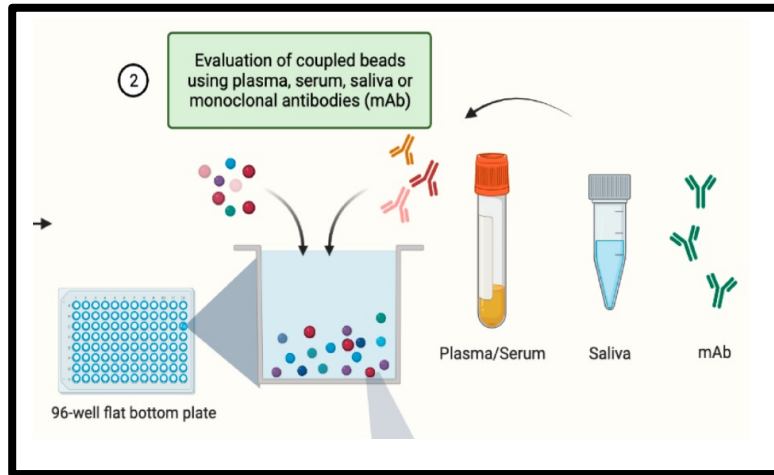
How you label your plate for your own reference will be different to how the plate NEEDS to be labelled for R.

Things to consider when setting up cohort:

- Antigen nomenclature – be consistent throughout study

- Bead IDs used for each antigen – again try and stay consistent throughout study

- Determine study size and make enough bead/antigen coupling at the beginning

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Naïve 1/100	Pos. 1/50	Pos. 1/100	Pos. 1/200	Pos. 1/400	Pos. 1/800	Pos. 1/1600	Pos. 1/3200	Pos. 1/6400	Pos. 1/12800	Pos. 1/25600
B	Blank											
C												
D												
E												
F												
G												
H												

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The assay – IgG Ab protocol

Step One
Dispense capture beads
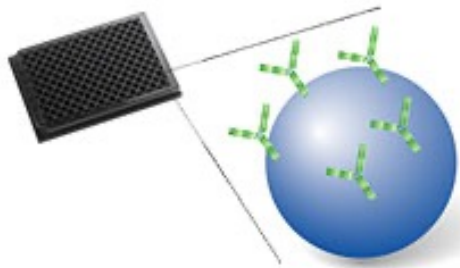
Step Two
Add samples
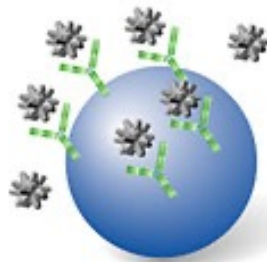
Step Three
Add detection antibody
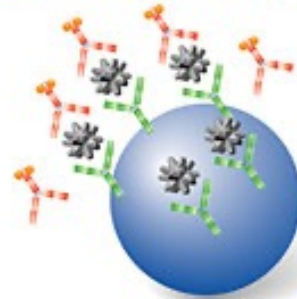
Step Four
Add reporter dye
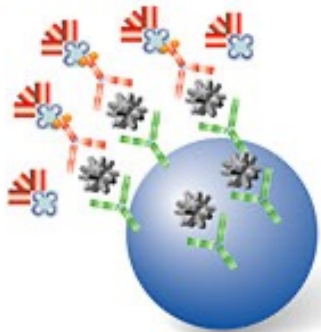
Step Five
Fluorescent sorting and data reduction

Classification laser

Reporter laser
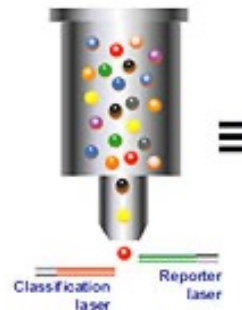
1. Beads conjugated to antigen are dispensed into the appropriate wells

2. Samples are added in singlicate. Standard curve is added as serial dilution. Incubate RT in the dark

3. Wash unbound antibodies

4. Detector antibody added. In our assay the detector antibody is already coupled to a reporter dye so steps 3 and 4 are combined. Incubate RT in the dark

5. Wash unbound antibodies and resuspend ready for magpix

The assay – at the machine
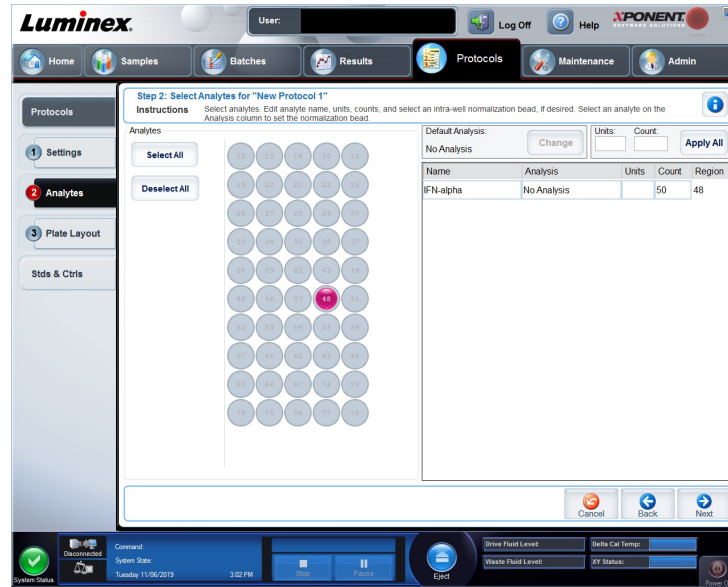
Prior to putting your plate on the machine, you need to ensure the machine is on and primed for running:

- Enhanced startup protocol

- Adjust probe height

- Calibrate system with kit

- Verify system with kit

- Ensure wash buffer and waste levels are ok

- Pick bead regions

- Setup protocol no controls

- *Only use blank and unknown buttons for 'in house' assay's*

The assay – raw data

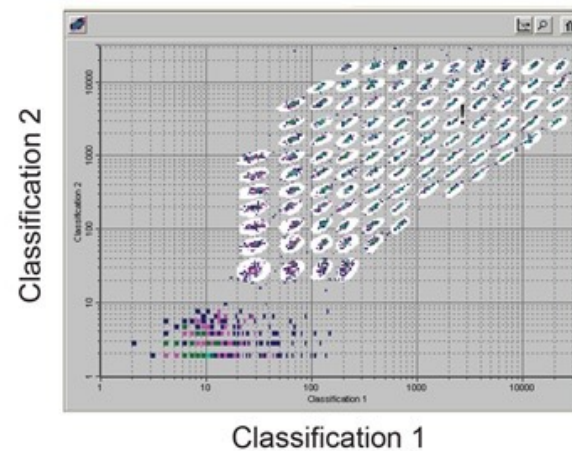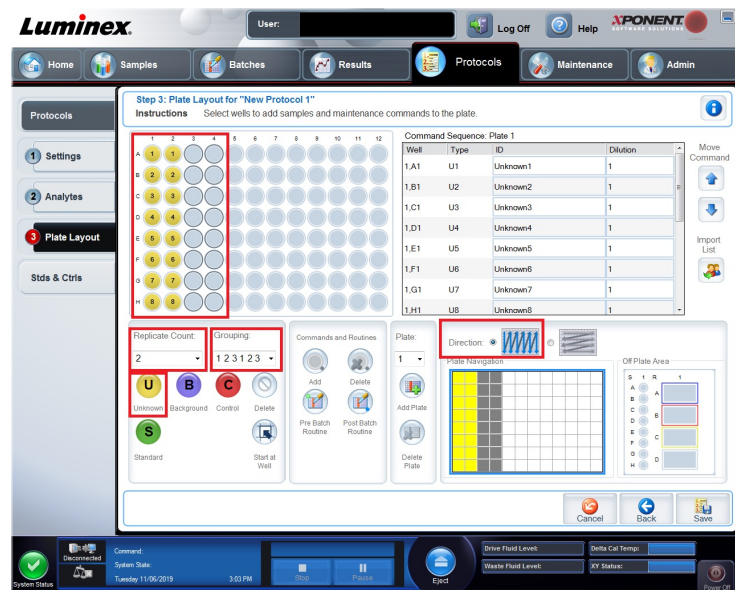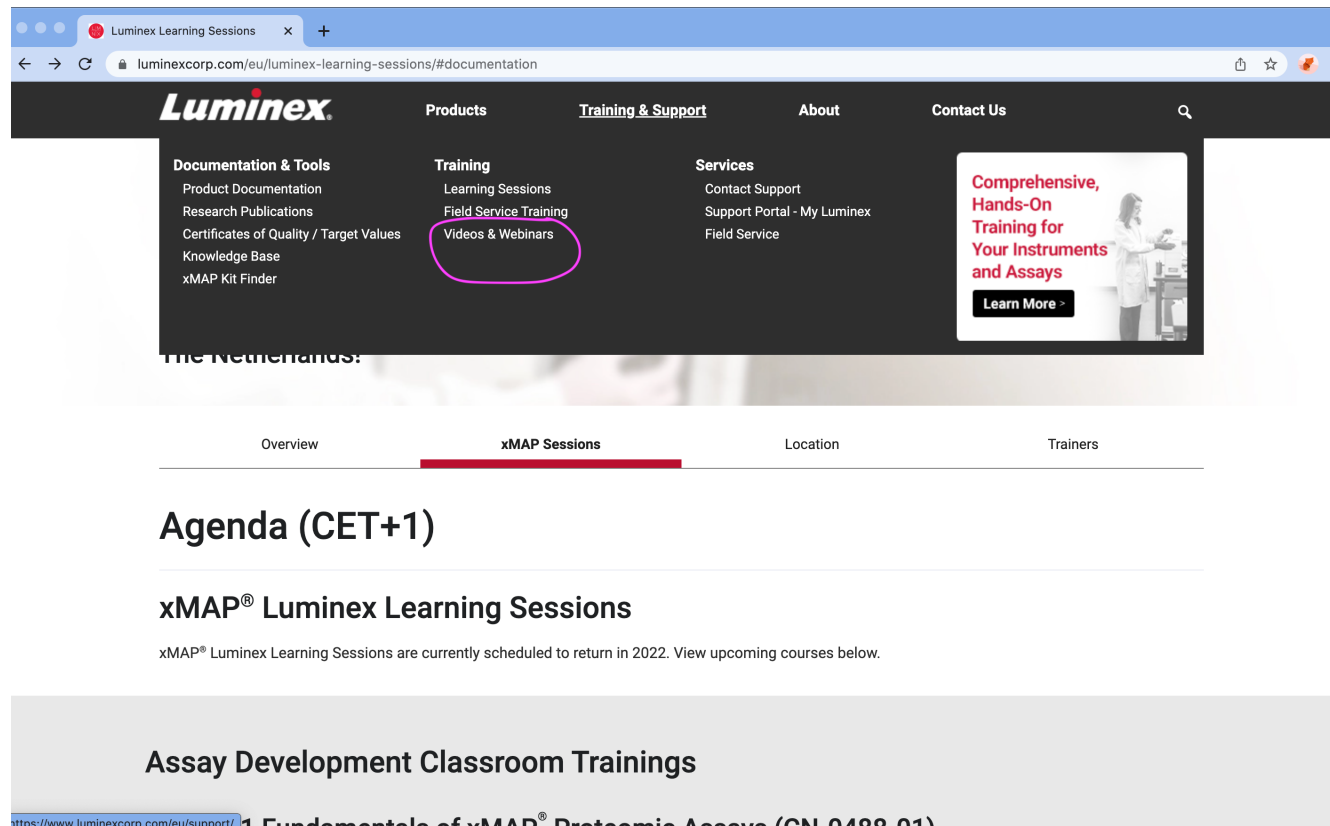|	A	B	C	D	E	F	G	H	I	J	K	L	M	N
63	DataType:	Median												
64	Location	Sample	IVB-Vict_21	Tetox_26	CS1H-NA_28	CS2H-NA_45	CNPH-Ac_46	CRBDH-WT_57	CSpikeH_62	NL63NPEc_65	OC43_66	229ES1H_77	HKU1Spike_78	Total Events
65	1(1,A1)	Blank 1	12	11	58	43	7	13	8	14	13	16	14	916
66	2(1,A2)	unknown1	2168.5	4388	133.5	129	242	116.5	123	611	1228	1792	1877.5	1143
67	3(1,A3)	S1	554	5194	4619.5	3899.5	11352	5815	10318	203	5956	2935.5	7591	868
68	4(1,A4)	S2	285.5	2995	2850	2768	10029	4448	8065	103	4941.5	1718	5226	986
69	5(1,A5)	S3	157	1748	1734.5	1828	8388	2801	5578.5	59	3645	927	3055	1080
70	6(1,A6)	S4	82	1015.5	947	1060	6550	1623	3124	39	2389	494	1630	984
71	7(1,A7)	S5	53	611.5	549	633	5135	963	1884	29	1517	268.5	968	1173
72	8(1,A8)	S6	33	287	300.5	372.5	3607	463	1011	22	882	147	510	869
73	9(1,A9)	S7	22	158.5	175	227	1849.5	209	520	17	469.5	82	263	1029
74	10(1,A10)	S8	17	99	115	137.5	1088	118	265	17	259	48	138.5	980
75	11(1,A11)	S9	15	50	87	89	559	62	127	16	148	32	78	1076
76	12(1,A12)	S10	14	33	71.5	63	308	36	66	15	79	25	48	1227
77	13(1,B1)	Blank 2	11	12	64	38	8	14	9	14	14	16	13	918
78	14(1,B2)	Unknown1	128.5	63.5	71	63.5	41	42	23	262	115	110	38	892
79	15(1,B3)	Unknown2	182.5	46	66	59	34	37.5	24	196	255	123	74	830
80	16(1,B4)	Unknown3	96	103	69	60.5	34	41	23	283	261	248	208	790
81	17(1,B5)	Unknown4	55	104	68	62	36	36	20.5	208	51	49.5	45	950
82	18(1,B6)	Unknown5	59	169.5	68	62	32	38	24	207	112.5	307	93	884
83	19(1,B7)	Unknown6	231	39	65	76	41	34	21	215.5	424	137	134	1060
84	20(1,B8)	Unknown7	54	173	67	72	85	47	28	369.5	228	154	255	1060
85	21(1,B9)	Unknown8	243	68	59.5	100	764.5	32	20	274.5	453	425.5	484	1020
86	22(1,B10)	Unknown9	125	43.5	64	125	176.5	33	38	254	267	236	307	821
87	23(1,B11)	Unknown10	76	376	67.5	62	1017	47	24	283.5	303	178.5	153	824
88	24(1,B12)	Unknown11	173	314	93	105.5	457.5	90.5	52	502.5	135	139	252	916
89	25(1,C1)	Unknown12	579.5	512	82	82	59.5	64.5	32	276	332	258.5	290	840
90	26(1,C2)	Unknown13	107	99	77	77	48	54	90	550	203	296	118.5	742
91	27(1,C3)	Unknown14	218	601	62.5	59	39.5	42	23.5	264	176	263	78.5	716
92	28(1,C4)	Unknown15	42	50.5	70.5	58.5	33	40	22	259	277.5	31	224	732
93	29(1,C5)	Unknown16	192	387.5	62.5	58	32	29	23.5	186	333	308	827	845
94	30(1,C6)	Unknown17	448.5	71	60	61	67	40	23	227	162	141	84.5	774
95	31(1,C7)	Unknown18	159.5	77	65	55	825.5	32	22	182	229	254	423.5	1048
96	32(1,C8)	Unknown19	421	77	64.5	57	34	35	21	183	218	152	418	887
97	33(1,C9)	Unknown20	239	269	62.5	57	32	34	29	190	246	346	272	907
98	34(1,C10)	Unknown21	106	81.5	62	67	51	49	26	371.5	165	117	379.5	949
99	35(1,C11)	Unknown22	126.5	419	63	49	22	25.5	14	131.5	147	242.5	206	741
100	36(1,C12)	Unknown23	180	36.5	62	58	24	30	22	196	341	118	233	923
101	37(1,D1)	Unknown24	102	85	74	63	44	37	24	203	97.5	106	176	901
102	38(1,D2)	Unknown25	165	126.5	66	61.5	31.5	37	26	206	174	104.5	139	720
103	39(1,D3)	Unknown26	126	115	72	68	58	49	44	348	546.5	656	312	599
104	40(1,D4)	Unknown27	95	442.5	73.5	82.5	722	59	37.5	741	186	111	73	793
105	41(1,D5)	Unknown28	97	432.5	75	85	636.5	61	33	734	189	118	76	831
106	42(1,D6)	Unknown29	137	1663	58	50	26	29.5	17	173.5	110	98	111	748

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Plate	1	2	3	4	5	6	7	8	9	10	11	12
2	A	Blank 1	Control	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
3	B	Blank 2											
4	C												
5	D												
6	E												
7	F												
8	G												
9	H												
10													

Results should be saved as an excel document

- **Keep nomenclature consistent throughout. Start as you mean to continue**

- **Ensure no spaces in antigen names**

- **If running multiple blanks, ensure they are numbered sequentially**

⑤ Analysis of detected MFIs for each bead region

Thank you - Questions

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https://www.luminexcorp.com/eu/luminex-learning-sessions/#documentation