

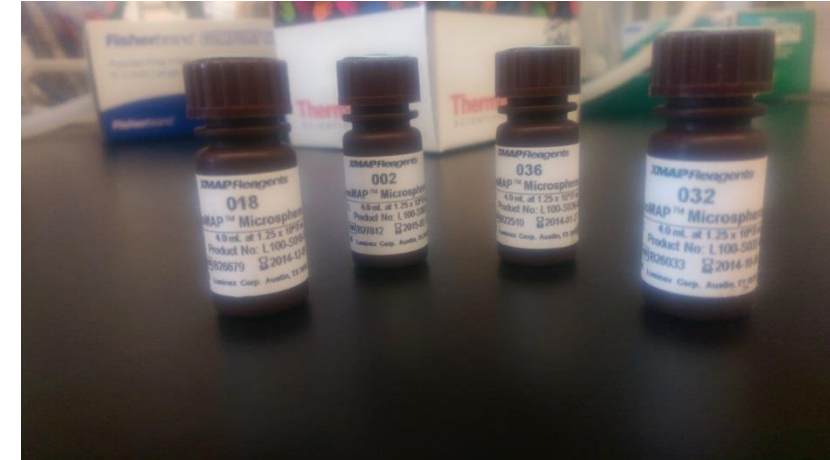
# TECHNICAL ASPECTS OF MULTIPLEX-BASED SERO SURVEILLANCE

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# Principles of multiplex-based serology

- Bead-based antibody detection
- Detects antibodies against multiple antigens (and therefore multiple pathogens) in a single well
- Each bead set is internally dyed to give it a unique spectral address
- Each bead set is chemically conjugated to an antigen of interest
- Fluorescent read-out for antibody binding to antigen on bead



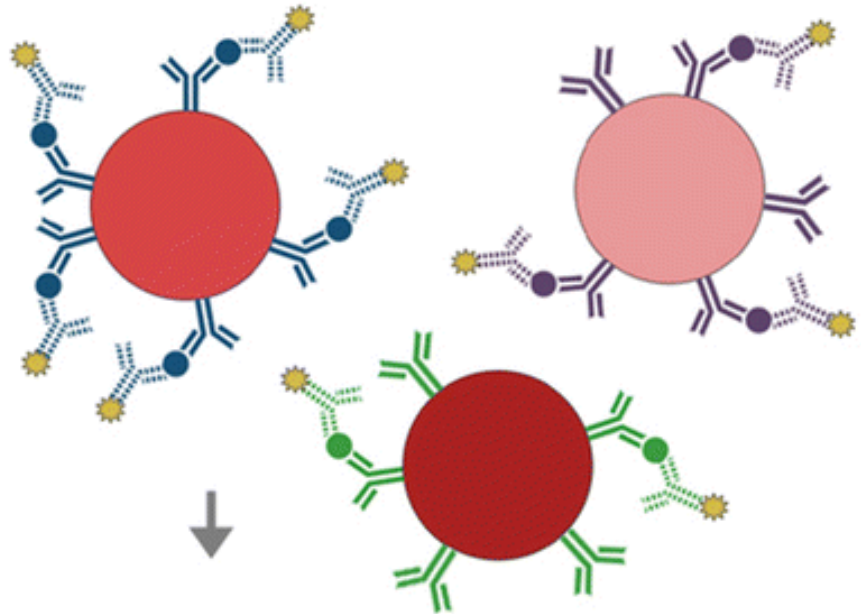
# Advantages of multiplex





- Low sample volume
- Minimize technician time compared to running multiple individual ELISAs
- Broad dynamic range of readout
- High “N” per well
- Output:
  - 160 samples (2 plates)/day/technician
  - 3 days/week = 480 samples/week/technician
  - 3000 samples = 6.25 weeks



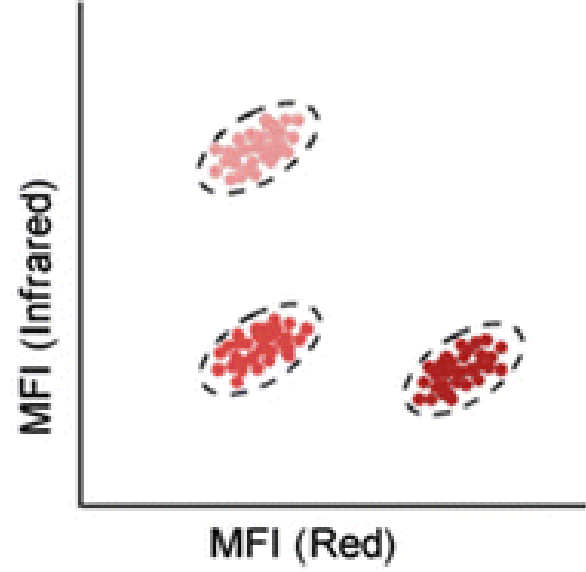
# Multiplex bead array

BEAD COMPLEXES AFTER SAMPLE INCUBATION

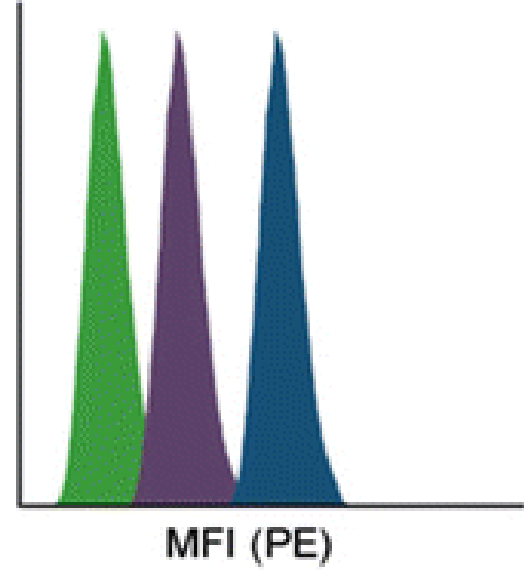


-  Capture antibody
-  Biotinylated detection antibody
-  Cytokine/chemokine
-  Streptavidin-PE


IDENTIFICATION OF BEAD REGION

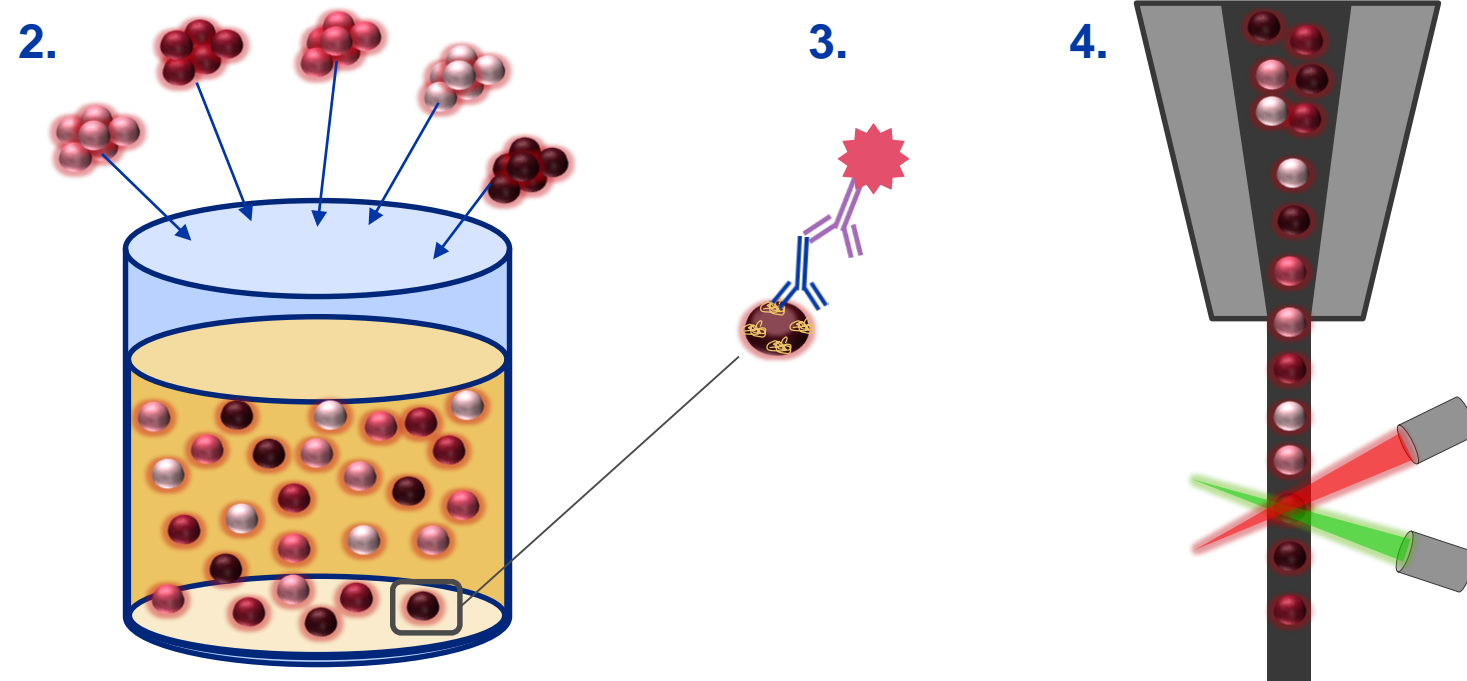


PE SIGNAL ASSOCIATED WITH BEAD



# Example of multiplex panel and assay

1. **NIE** (Strongyloidiasis)   
**T24H** (Cysticercosis)   
**Bm14** (Lymphatic filariasis)   
**Bm33** (Lymphatic filariasis)   
**Wb123** (Lymphatic filariasis) 

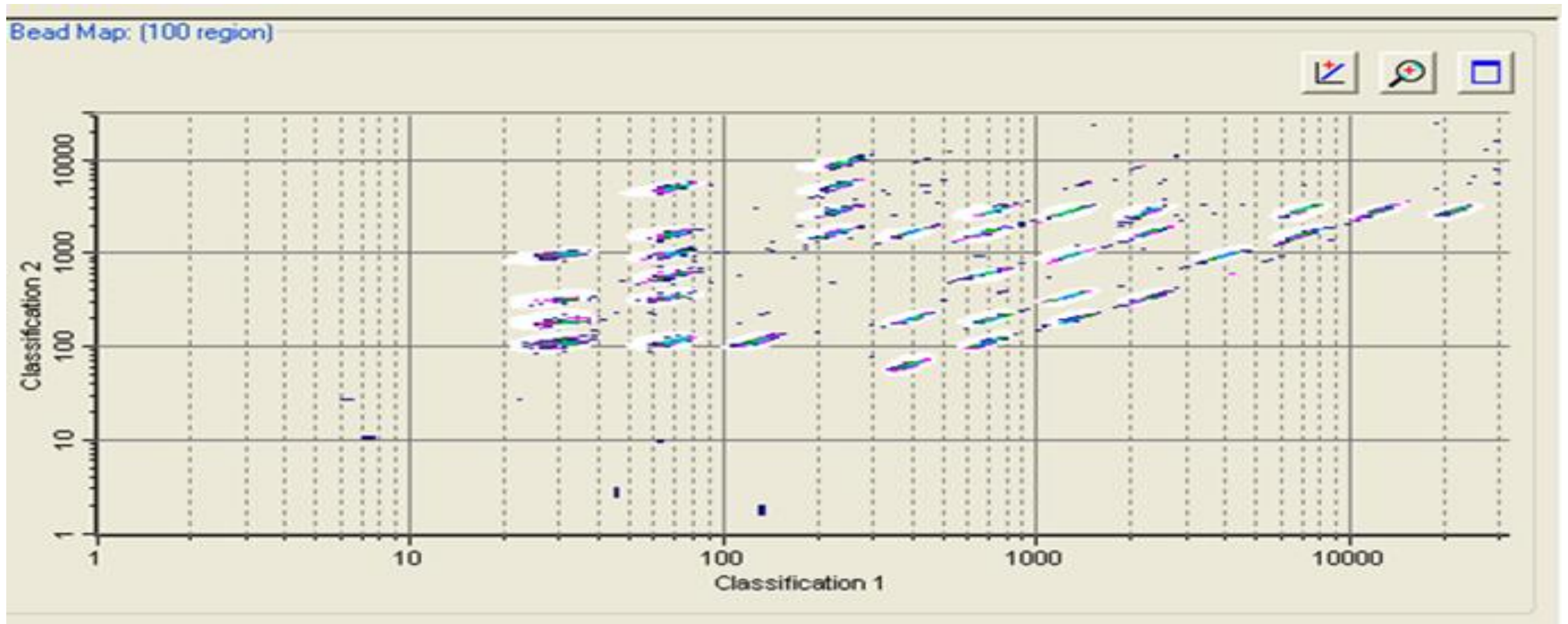


1. Panel of antigens is selected and antigens are chemically bound to beads

2. Beads are mixed together in well and sample added.
3. Anti-human detection antibody with fluorescent tag binds antibody bound to antigen on bead
4. Sample is run through flow cytometer (Luminex or MagPix) and interrogated by laser.



# Screen shot of bead fluorescence on Luminex



# Readout: MFI data exported to Excel: sample (row) by antigen (column)

MV-N (24)	Bm14/GST (52)	TetanTox (21)	Bm33/GST (64)	NIE/GST (70)	SAG2/GST (50)	MSP-1(19)[3D7]/G ST (23)	MSP-1(42) (3D7) (60)	MSP-1(42) (FVO) (72)	Dengue 2 VLP (80)	Dengue 3 VLP (88)	T24H (57)	P.vivax 1(19)/GST (2)	MSP- ChikVirus E1 (12)	JEV CL F8 (74)	C2 (79)	WN VLP CL (97)	YF VLP CL 2D3 (93)	YF VLP CL 1F3 (97)	GST (68)	Wb123 (41)
FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd	FI - Bkgd
2324	13	25539	9	23753	100	2637	2214	1388	24752	27498	111	37	805	2737	1687	198	272	13	170	
1074	21	24423	59	24397	21	239	270	57	24815	26396	97	111	962	2613	1738	921	1182	55	76	
2869	7	1578	6	24585	7	488	571	376	22601	25404	162	168	62	2068	924	207	268	7	34	
1405	5	27563	13	29302	6	27921	28612	27195	27058	28750	69	43	117	4673	3397	412	307	8	25	
4807	9	25497	6	1465	5	22	56	36	27217	28746	60	37	247	3205	1851	295	246	13	17	
1044	30	26244	5	25249	0	11	33	38	25796	28009	73	8	281	3098	2115	300	196	1	47	
7211	4	25687	4	219	4	37	42	58	25677	28548	41	35	35	2997	1927	431	272	13	10	
4953	16	147	18	27826	5	11	29	25	24886	27096	108	10	3399	4451	2561	395	522	9	86	
4138	2153	26851	49	6186	5	182	1879	110	25699	27598	127	61	26978	3165	2084	186	177	16	12	
4326	4	26877	7	163	0	5	90	4	24282	26723	18	6	37	1184	589	133	107	6	80	
3881	8	27104	6	22	3	5	12	13	23344	25246	9	5	24427	1339	850	162	212	7	11	
3649	27	26787	9	14589	27	19	89	69	25630	28206	58	32	255	1696	1249	271	418	14	277	
6765	475	27033	458	27902	7	31	103	43	18001	24590	42	19	1291	1349	537	208	307	8	75	
3098	22	9278	9	2563	0	13	37	10	26092	28479	57	791	3673	4430	2624	218	125	7	18	
2314	18	11542	8	26601	10	126	155	72	25585	27796	36	170	463	2985	1810	342	424	11	72	
6323	15	27089	41	25090	66	3915	4443	7946	26660	28653	27	106	17794	4968	3525	990	823	7	26	
5523	14	26788	10	9073	6	618	646	284	24826	26855	81	22	22890	2850	2339	347	402	6	80	
7259	9	26883	36	28441	3	30	88	28	26445	27861	59	6	215	3592	2543	250	275	47	42	
6780	53	27871	130	27392	41	390	424	285	26912	28482	721	595	5774	3900	2707	481	372	6	150	
6107	8	28200	9	262	4	6400	6220	1032	26938	28726	26	127	26576	3548	2057	135	178	10	21	
1313	11	28910	23	12801	8	16	31	12	24005	27039	46	5	16239	1046	658	181	207	7	9	
1378	2226	27016	57	2858	1	116	1700	111	26172	27612	39	12	14372	2655	1605	365	369	24	20	
1227	7	27490	6	23793	13	9	39	29	27403	29119	144	5	28297	4298	2812	286	192	5	51	
432	5	26250	21	6056	10	7	17	13	25783	27736	14	21	70	2577	1786	284	194	19	17	
1990	2	27104	14	1399	3	15	81	12	25585	27640	152	18	16484	2805	1370	505	462	7	30	
1917	2	26629	4	29	4611	11	20	10	25129	27070	17	2	28013	2415	1398	110	136	3	8	
1370	2	28014	11	38	3	12	24	15	26069	28208	29	4	2875	3414	1227	185	166	5	32	
4437	6	26226	5	1295	5	39	95	21	25540	27591	67	24	1009	2562	1511	132	235	4	37	

# Antigens available for multiplex: Neglected Tropical Diseases

<b>Disease</b>	<b>Pathogen</b>	<b>Antigens</b>
Lymphatic filariasis (LF)	<i>Brugia malayi</i> <i>Wuchereria bancrofti</i>	Bm14, Bm33 Wb123
Onchocerciasis (River Blindness)	<i>Oncocherca volvulus</i>	Ov16
Trachoma	<i>Chlamydia trachomatis</i>	Pgp3, CT694
Yaws	<i>Treponema pallidum pertenue</i>	TmpA, rp17
Schistosomiasis	<i>Schistosoma mansoni</i>	SEA
Strongyloidiasis	<i>Strongyloides stercoralis</i>	NIE
Cysticercosis	<i>Taenia solium</i>	T24H
American trypanosomiasis	<i>Trypanosoma cruzi</i>	**
Leishmaniasis	<i>Leishmanis spp</i>	**



# Antigens available for multiplex: Vector-borne

<b>Disease</b>	<b>Pathogen</b>	<b>Antigens</b>
Malaria	<i>Plasmodium falciparum</i> <i>Plasmodium vivax</i>	MSP-1, MSP-2
Babesiosis	<i>Babesia microti</i>	Bm
Zika	Zika virus	
Chikungunya	Chikungunya virus	Chik
Dengue**	Dengue virus	VLP2 VLP3
Rift Valley Fever**	RVF virus	
Japanese encephalitis	JE virus	JEV CLF8
West Nile	WN virus	VLP CL C2
Yellow Fever	Yellow Fever Virust	VLP CL 1F3

# Antigens available for multiplex: Waterborne/Foodborne

<b>Disease</b>	<b>Pathogen</b>	<b>Antigens</b>
Cryptosporidiosis	<i>Cryptosporidium parvum</i>	Cp17 Cp23
Giardia	<i>Giardia lamblia</i>	VSP3 VSP5
Toxoplasmosis	<i>Toxoplasma gondii</i>	Sag2
Amoebiasis	<i>Entamoeba histolytica</i>	Lip32
Salmonella LPS Groups and D	<i>Salmonella typhimurium</i> <i>Salmonella enteritidis</i>	S typ B S ent D
Norovirus	Norovirus	
Streptococcus	<i>Streptococcus pylori</i>	SPEA SPEB
Campylobacter	<i>Campylobacter pylori</i>	Campy p18 Campy p39

# Example Panel: Congenitally Transmitted Diseases

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<b>Disease</b>
HIV
HepC
Syphilis
Herpes
Rubella
Toxoplasmosis

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# Why are some antigens not available on multiplex?

- The protein is antigenic and works in ELISA but will not chemically couple to bead (HepB Ag)
- Difficult to produce antigen
  - Recombinant protein may be difficult to express e.g. in insoluble fraction of bacterial culture (CPAF)
  - Native proteins may be limited in availability or lot-to-lot variation (SEA)
- No good positive and negative control panels
- The antigen lacks sensitivity
- The antigen lacks specificity
  - Dengue antigens cross-react with other arboviruses
  - Scabies antigens x-react with house dust mite
  - Babesia antigens x-react with malaria antigens

# Analyzing Serology Data

- Determining positive and negative cutoffs
  - Positive and negative panels
- Understanding what responses to each antigen means
  - Vaccination v natural infection?
  - Current v historical infection?
  - Understand the natural history of each disease – work with subject matter experts
  - Does response against a single antigen imply positivity or need multiple
- Need strong collaboration with subject matter experts for sampling strategies and data interpretation.

## Limitations to multiplexing

- Cannot separate out isotype-specific antibodies on multiplex
  - e.g. IgM-specific responses need to be measured separately
  - Total IgG v IgG4
- Concentration of specific antibody in serum may need different dilutions
  - We use 1:400 dilutions of serum in our multiplex assay
  - Collaboration with HIV group on maternal-child health testing to use 1:100
- Need exceptional quality control and data management



# Challenges for Multiplex in Low-to-Middle Resource Countries

- Need for a continuous, reliable power supply
- Running water – access to distilled water
- Temperature controlled room
- Refrigerator and freezer
- Dedicated computer
- Biosafety cabinet (preferred)
- Sufficient funds for equipment maintenance and purchase of sheath fluid/ other consumables
  - Accessibility of technical support from instrument companies
- Coupling the antigen to the bead