

Immunoproteomic analysis of lymphocyte secretion fluid in patients with acute phase typhoid fever in Bangladesh

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Lymphocyte secretion assay: TPTest

ALS: antibody in lymphocyte supernatant

- 1 cc blood
- Buffy coat or PBMCs.
- Culture 37C 18-48 hours in media, unstimulated
- Recover supernatant
- Can use in ELISA or dot blot assays

- These cells reflect “in vivo” stimulated cells
- They are acute phase
- Could consider this analysis an “antibody amplification assay”

Antigen-Specific Immunoglobulin A Antibodies Secreted from Circulating B Cells Are an Effective Marker for Recent Local Immune Responses in Patients with Cholera: Comparison to Antibody-Secreting Cell Responses and Other Immunological Markers

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Gut-derived lymphocytes transiently migrate through the peripheral circulation before homing back to mucosal sites and can be detected using an ELISPOT-based antibody secreting cell (ASC) assay. Alternatively, transiently circulating lymphocytes may be cultured in vitro

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Salmonella enterica Serovar Typhi-Specific Immunoglobulin A Antibody Responses in Plasma and Antibody in Lymphocyte Supernatant Specimens in Bangladeshi Patients with Suspected Typhoid Fever[∇]

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Many currently available diagnostic tests for typhoid fever lack sensitivity and/or specificity, especially in areas of the world where the disease is endemic. In order to identify a diagnostic test that better correlates with typhoid fever, we evaluated immune responses to *Salmonella enterica* serovar Typhi (serovar Typhi) in individuals with suspected typhoid fever in Dhaka, Bangladesh. We enrolled 112 individuals with suspected typhoid fever, cultured day 0 blood for serovar Typhi organisms, and performed Widal assays on days 0, 5, and 20. We

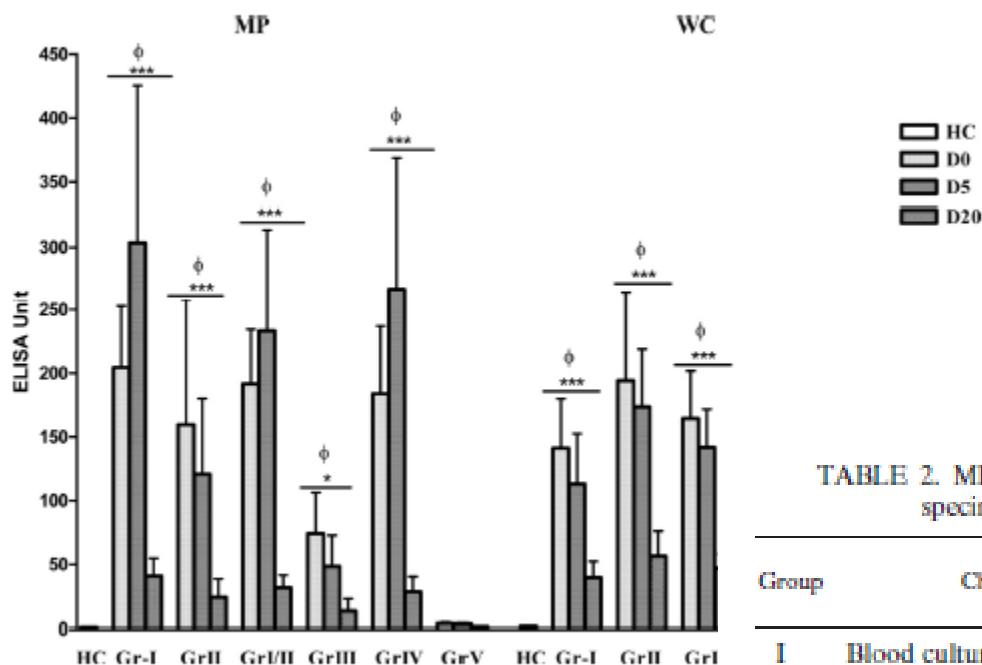


FIG. 5. MP- and WC-specific IgA responses in ALS specimens of different study groups on different days (D0, D5, and D20). Geometric means with the SEM are shown for day 0 (D0), day 5 (D5), and day 20 (D20). Statistical significance: $P < 0.0001$; **, $P < 0.005$; *, $P < 0.05$. Statistical difference compared to GrV: ϕ , $P < 0.05$.

TABLE 2. MP specific IgA responses in plasma and ALS specimens at the acute stage of disease^a

Group	Characteristics	Sample size	IgA response (%)	
			Plasma	ALS
I	Blood culture positive	26	21 (81)	26 (100)
II	Fourfold Widal titer change	13	8 (58)	13 (100)
III	≥ 320 Widal titer	41	18 (43)	29 (70)
IV	Culture negative, Widal negative but a >10 ELISA unit ALS response	14	4 (29)	14 (100)
V	Culture negative, Widal negative and a ≤ 10 ELISA unit ALS response	18	5 (28)	0 (0)

^a Analyses of specimens on study day 1 from patients subgrouped according to different clinical and immunological characteristics. Positive responses were calculated as $>GM$ plus two standard deviations of healthy control results; plasma MP-IgA response cutoff, ≥ 61 ELISA units; ALS MP-IgA response cutoff, >10 ELISA units.

ALS responses to MP (membrane preparation) fall by day 20

Protein Microarray

- Collaborators: Li Liang and Phil Felgner; UC Irvine
- Salmonella arrays contain ~ 2000 proteins including membrane proteins and others predicted by software to potentially be immunogenic
- Proteins are expressed in 96 well plates using E coli in vitro transcription and translation kits before being spotted on to a nitrocellulose-coated glass slides.

Samples

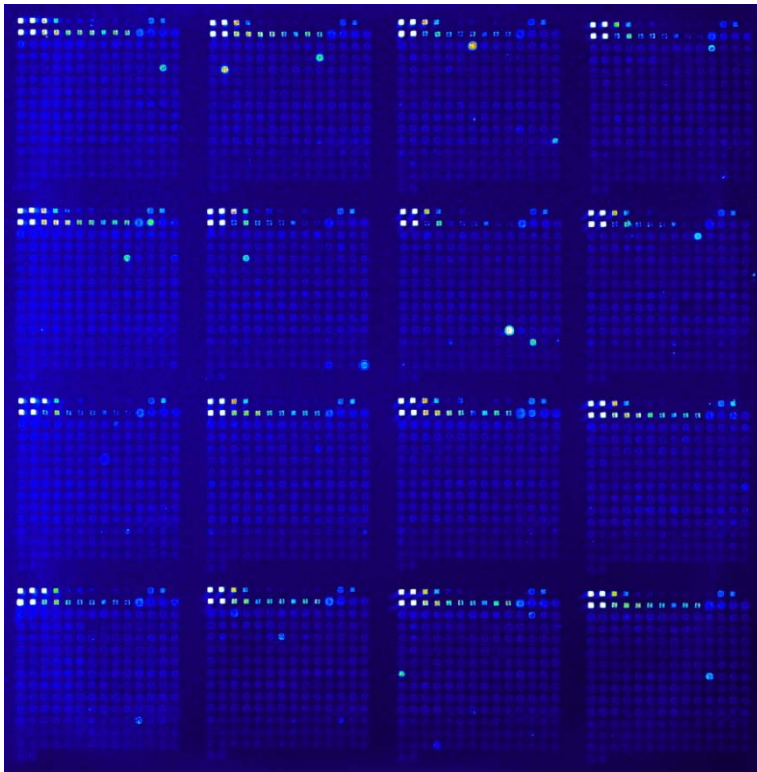
- 1) Typhoid Positive samples, n=10
- 2) Healthy controls, n=5
- 3) Other febrile illness, n=5

Each sample was probed with the following secondary Antibodies

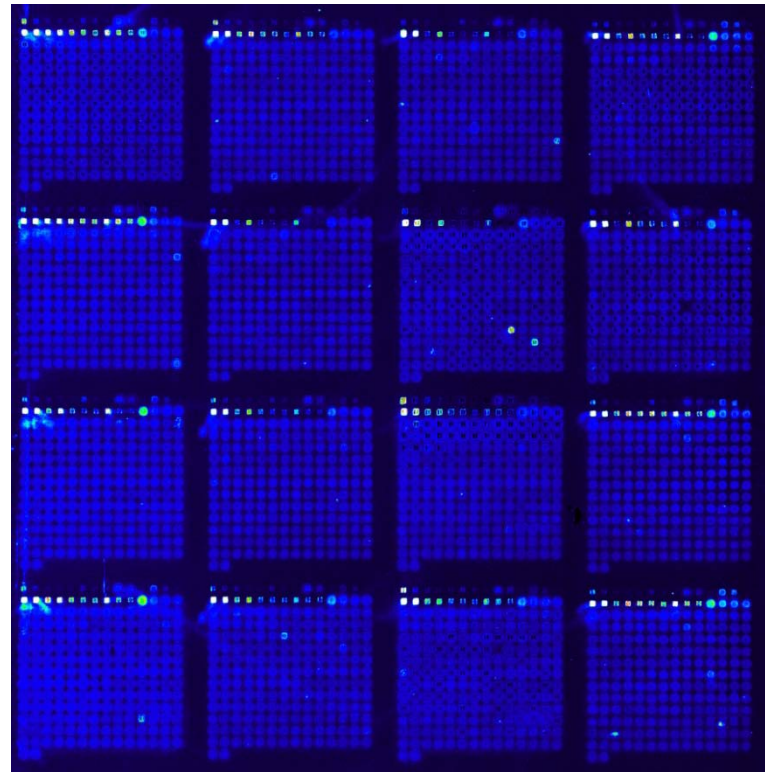
- 1) Biotin-conjugated α -Human IgA
- 2) Biotin-conjugated α -Human IgG

Typhi Patient (STFL-176)

Biotin- IgG



Biotin- IgA

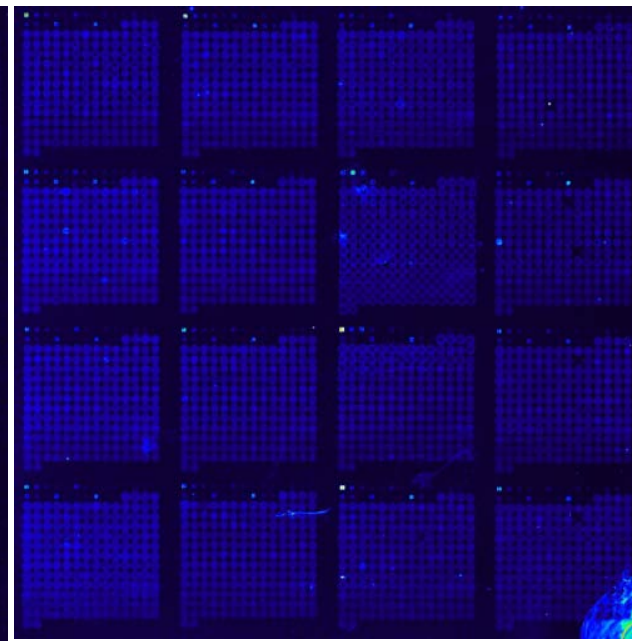
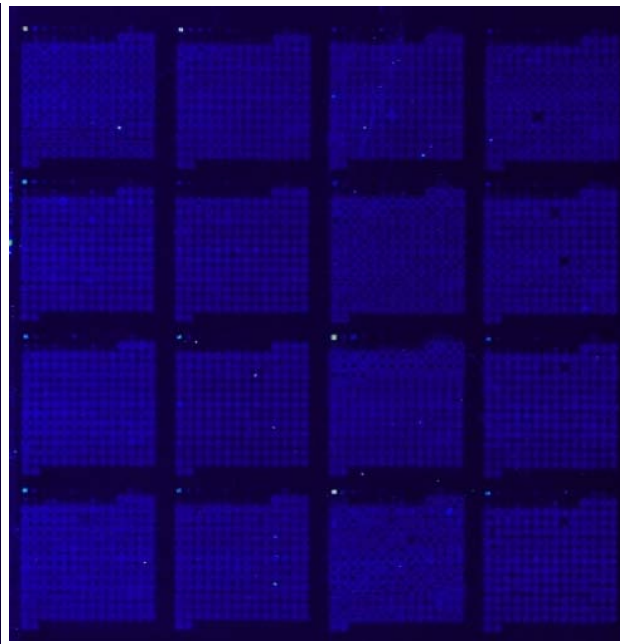
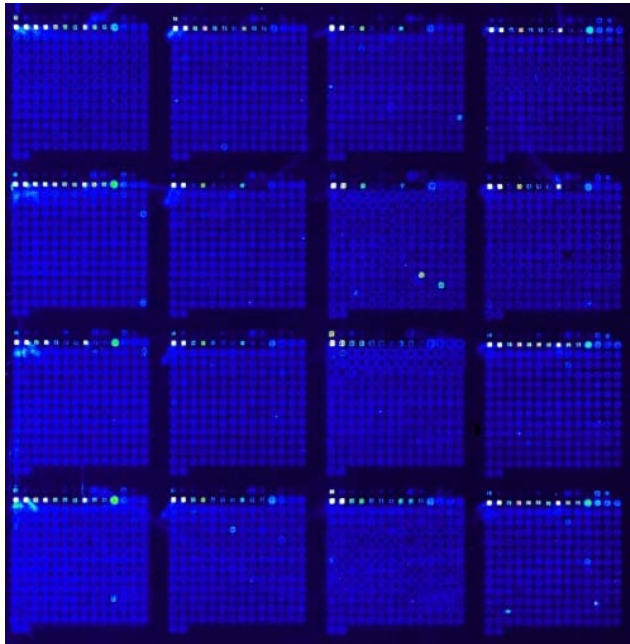


Biotin-IgA

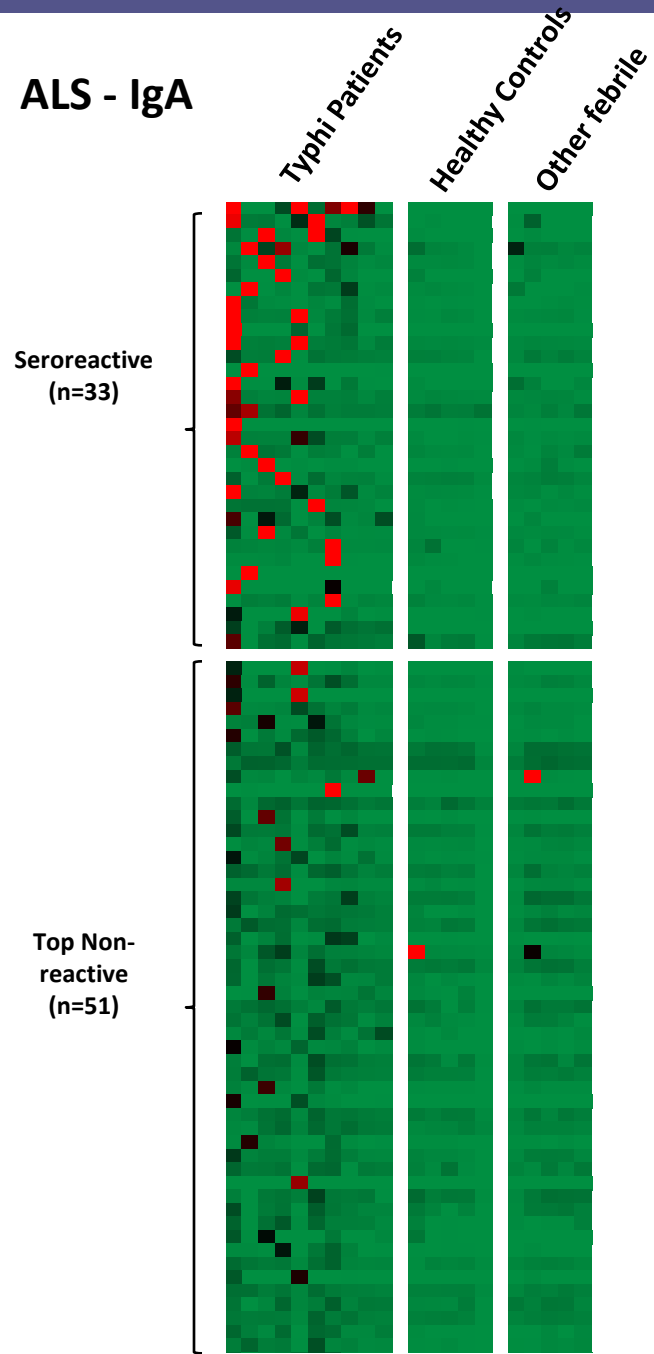
Typhi patient

Healthy control

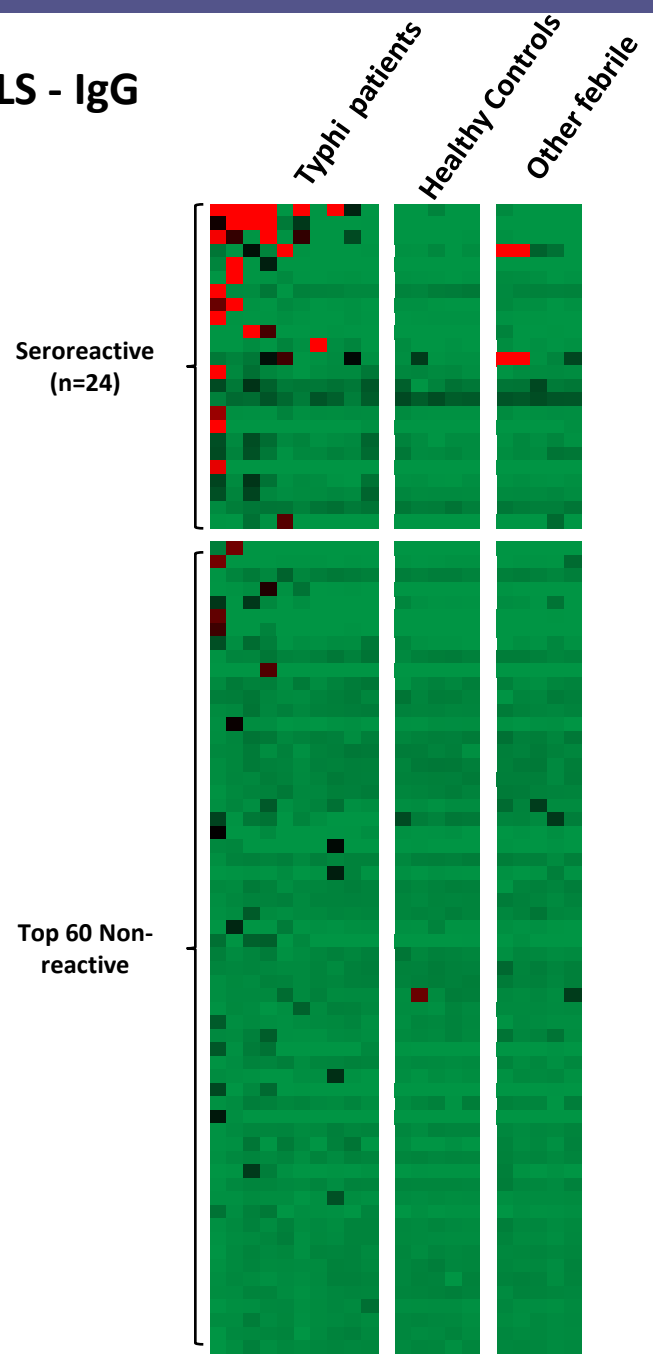
TB



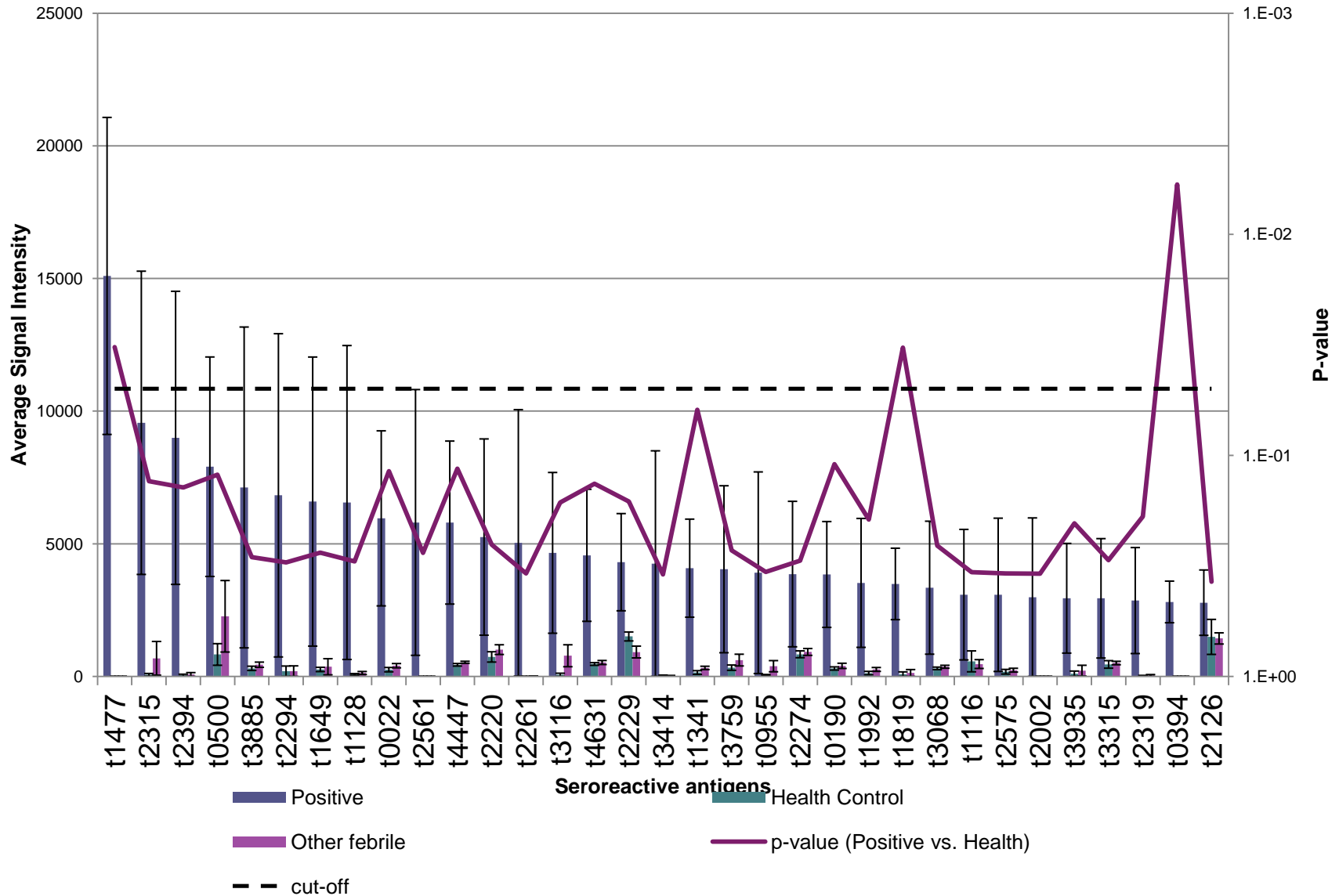
ALS - IgA



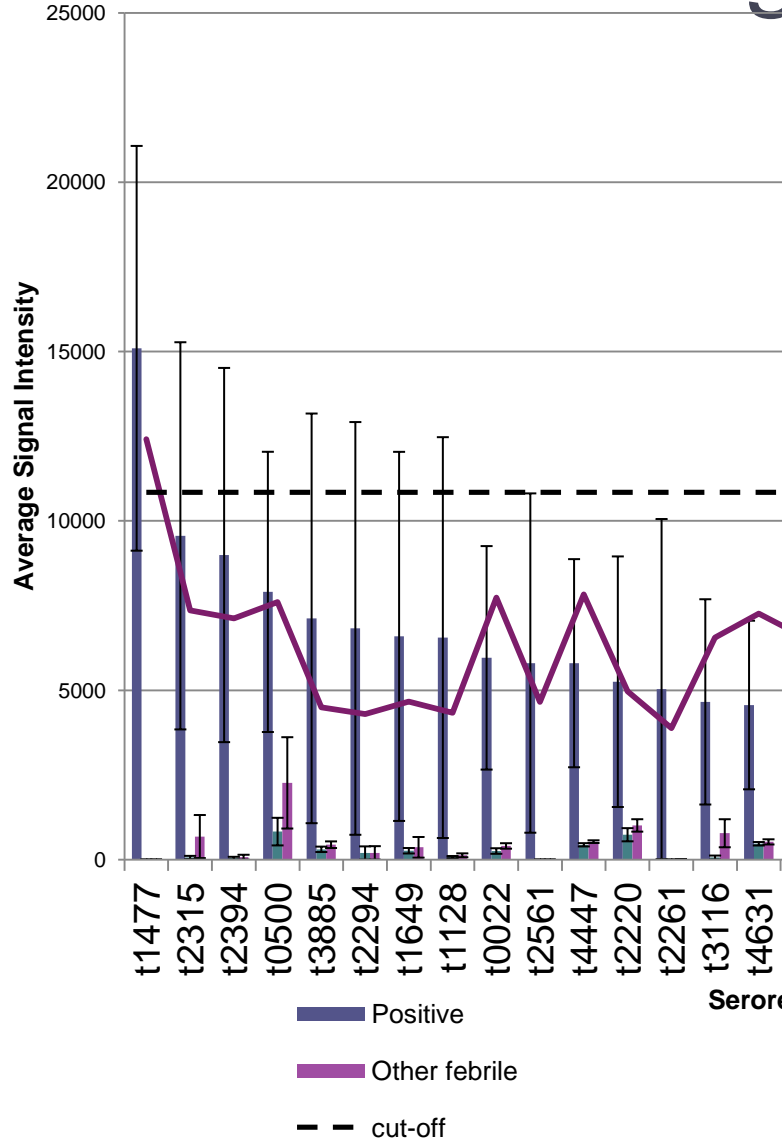
ALS - IgG



Seroreactive Antigens - IgA

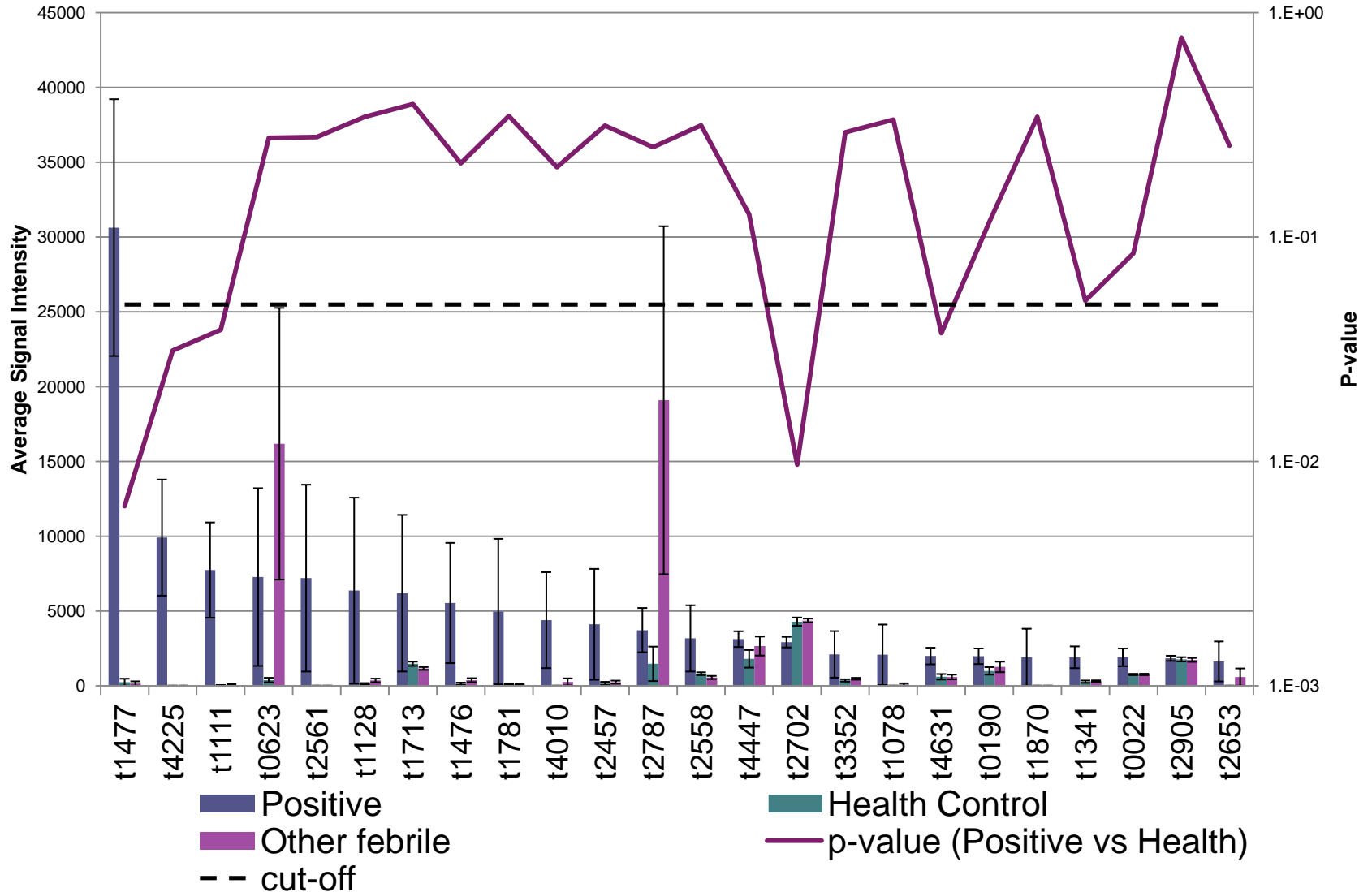


Seroreactive Antigens

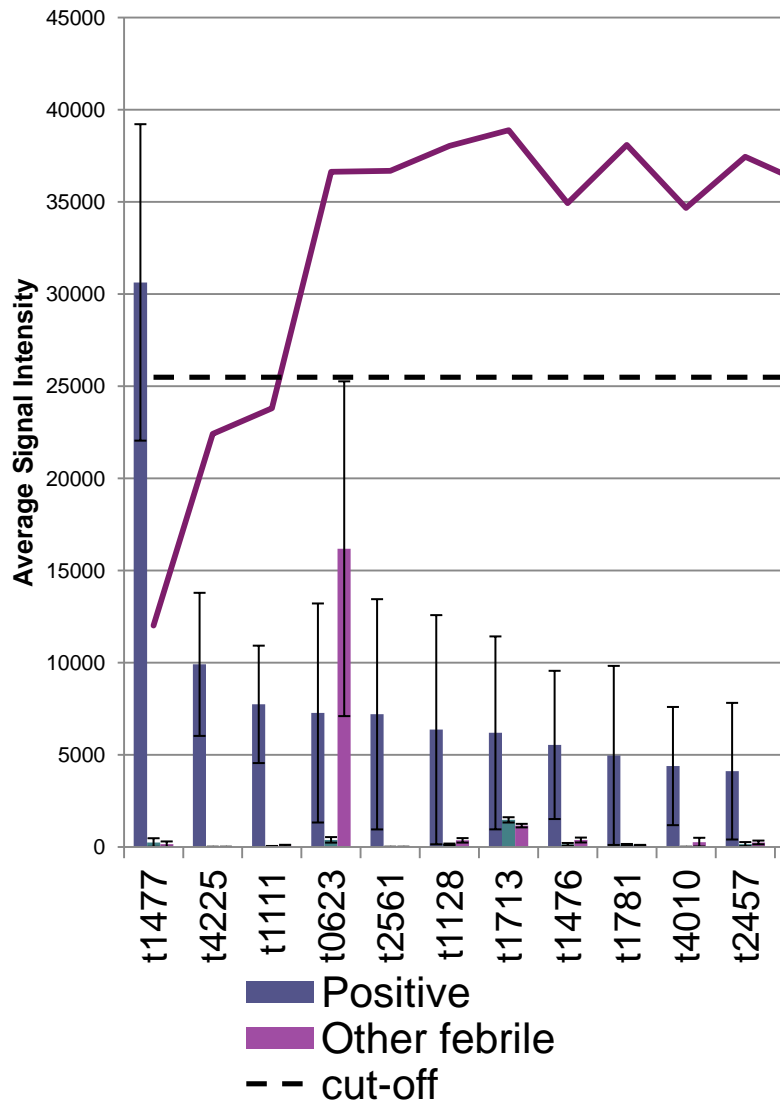


Locus	Gene	Function
t1477	hlyE	haemolysin HlyE
t2315	fimF	fimbria-like protein FimF precursor
t2394		conserved hypothetical lipoprotein
t0500	dedD	DedD protein
t3885	dppA	periplasmic dipeptide transport protein precursor
t2294		hypothetical protein
t1649	tonB	TonB protein
t1128		putative heat shock protein
t0022	bcfA	fimbrial subunit
t2561	safA	probable lipoprotein
t4447	ytfB	conserved hypothetical protein
t2220	rlpB	rare lipoprotein B precursor
t2261	dsbG	thiol:disulfide interchange protein DsbG precursor
t3116		conserved hypothetical protein
t4631	sthD	putative fimbrial subunit
t2229	rlpA	rare lipoprotein A
t3414		hypothetical protein

Seroreactive Antigens - IgG



Seroreactive Antigens in *Y. enterocolitica*



Locus	Gene	Function
t1477	hlyE	haemolysin HlyE
t4225	phoN	nonspecific acid phosphatase precursor
t1111	cdtB	putative toxin-like protein
t0623	sspH2	secreted effector protein
t2561	safA	probable lipoprotein
t1128		putative heat shock protein
t1713		putative lipoprotein
t1476		hypothetical protein
t1781	csgG	assembly/transport component in curli production
t4010	yrfG	putative hydrolase
t2457	yajC	putative membrane protein

p-value (Positive vs Healthy)

IgG

Positive control
Health

Other febrile

Acute

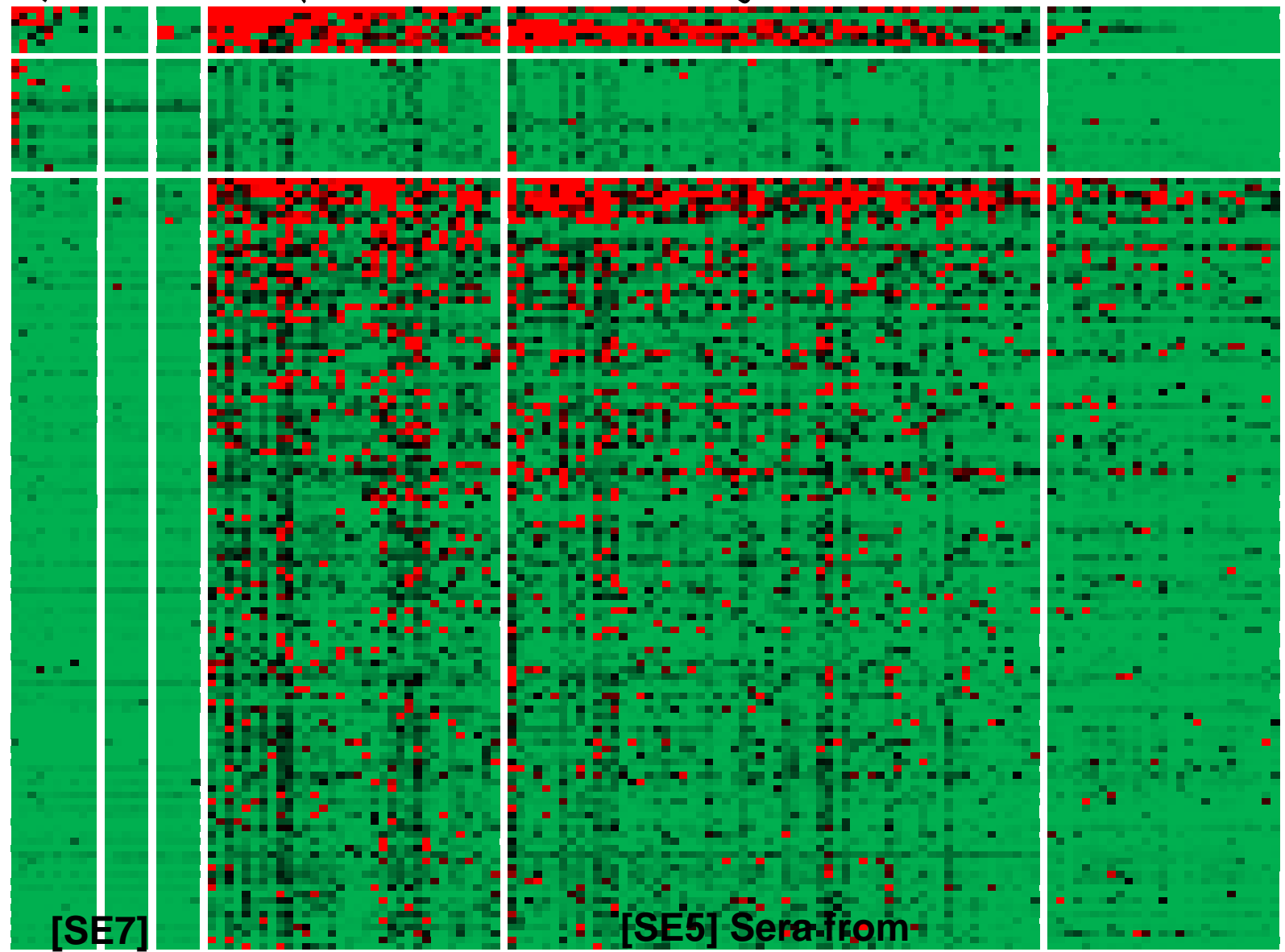
Uninfected

GCRC

Seroreactive in both studies (n=7)

Seroreactive for ALS only (n=17)

Seroreactive in Vietnam sera study only (n=117)

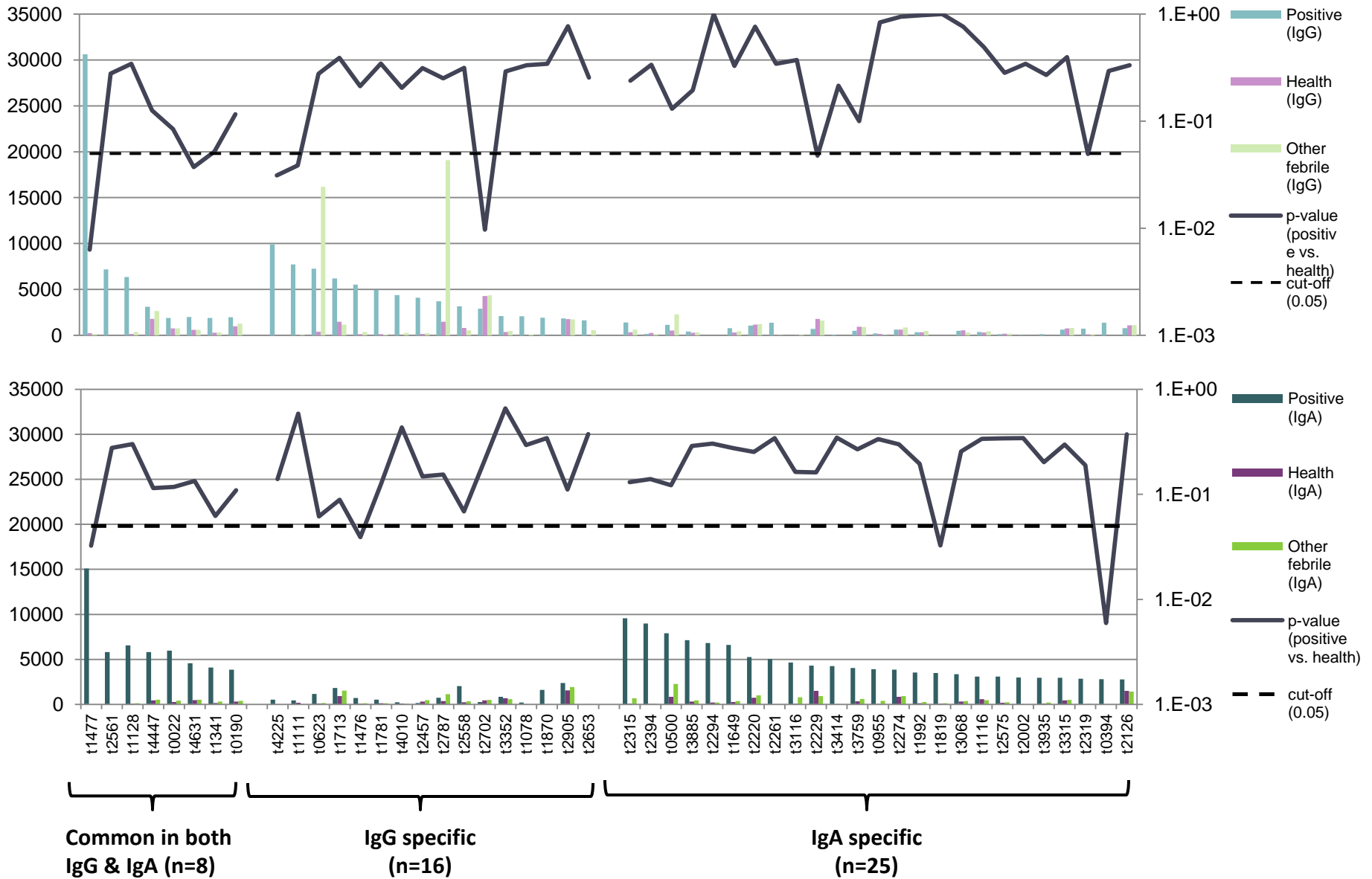


[SE7]
ALS

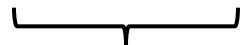
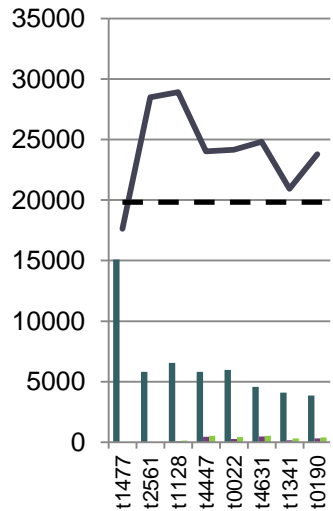
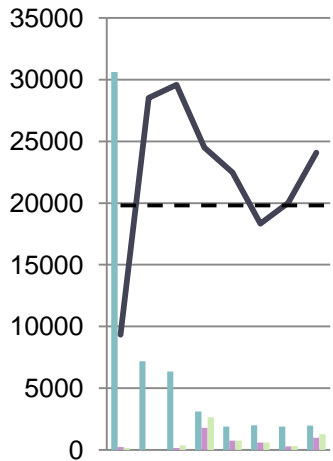
[SE5] Sera from
Vietnam

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Seroreactive antigens alignment between IgG & IgA



Seroreactive antigens Common to both IgG & IgA

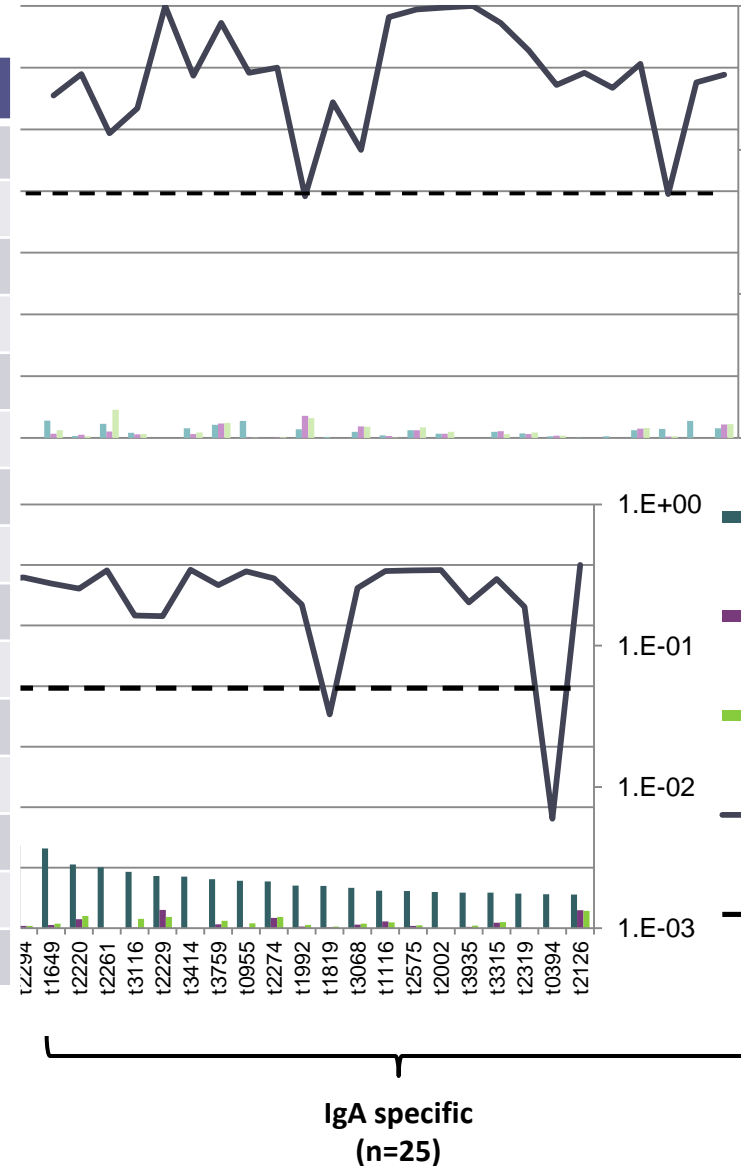


Common in both
IgG & IgA (n=8)

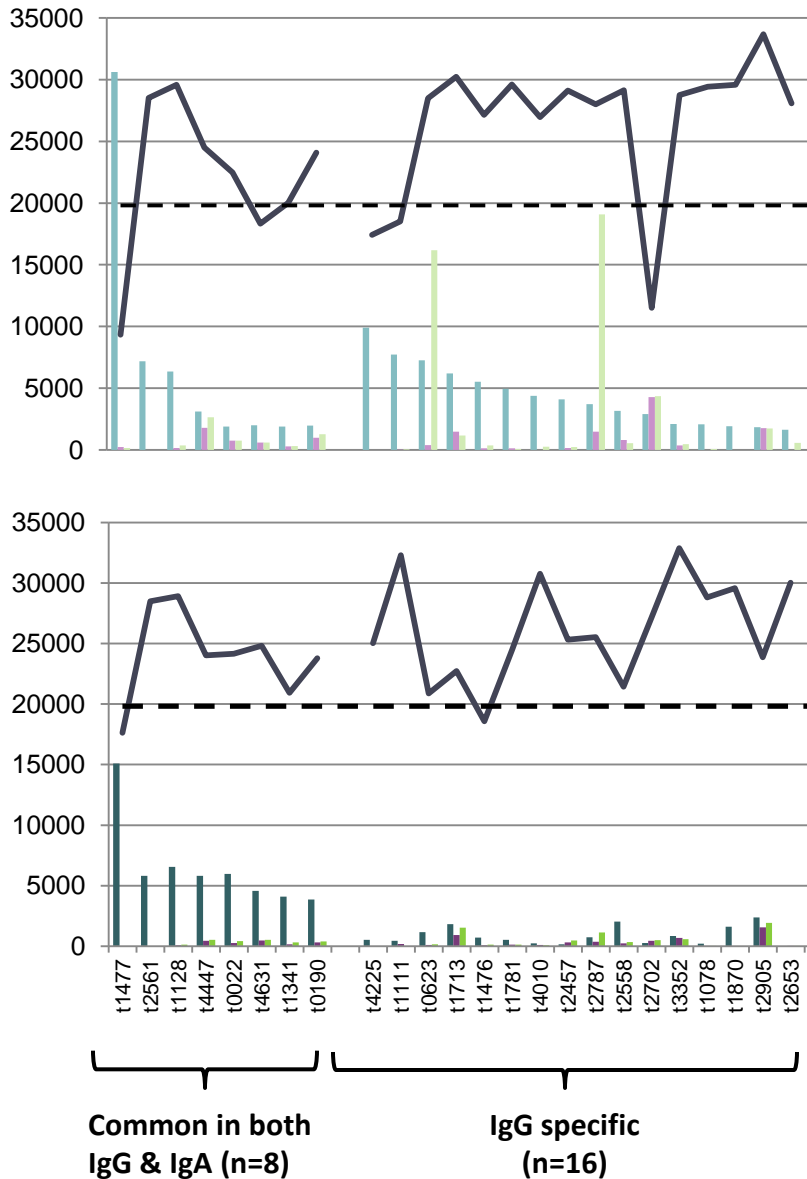
Ty2	CT18	LT2	Gene Name	Function
t1477	STY1498		hlyE	haemolysin HlyE
t2561	STY0332		safA	probable lipoprotein
t1128	STY1871	STM1251		putative heat shock protein conserved hypothetical protein
t4447	STY4752	STM4396	ytfB	
t0022	STY0024	STM0021	bcfA	fimbrial subunit
t4631	STY4940	STM4592	sthD	putative fimbrial subunit
t1341	STY1649	STM1473		outer membrane protein
t0190	STY0207		staA	putative fimbrial protein

Seroreactive antigens specific to IgA

Ty2	CT18	LT2	Gene Name	Function
T2315	STY0595	STM0548	fimF	fimbria-like protein FimF precursor
T2394	STY0509	STM0465		conserved hypothetical lipoprotein
T0500	STY2595	STM2364	dedD	DedD protein
T3885	STY4168	STM3630	dppA	periplasmic dipeptide transport protein precursor
T2294	STY0616	STM0567		hypothetical protein
T3414	STY3672			hypothetical protein
T3759	STY4029	STM3758	fidL	putative exported protein
T0955	STY2131	STM1922	motB	motility protein B
T2274	STY0638	STM0594	fepB	ferrienterobactin-binding periplasmic protein precursor
T1992	STY0937	STM0941	ybjY	putative exported protein
T1116	STY1880a	STM1244	pagD	putative outer membrane virulence protein
T2575	STY0314			probable secreted protein
T2319	STY0590	STM0544	fimI	Putative fimbrin-like protein fimI
T0394	STY2701	STM2465	eutN	Putative ethanolamine utilization protein EutN
T2126	STY0796	STM0750	ybgF	putative exported protein



Seroreactive antigens specific to IgG



Ty2	CT18	LT2	Gene Name	Function
t4225	STY4519	STM4319	phoN	nonspecific acid phosphatase precursor
t1111	STY1886		cdtB	putative toxin-like protein
t0623	STY2467	STM1051	sspH2	secreted effector protein
t1713	STY1247	STM1207		putative lipoprotein
t1476	STY1499			hypothetical protein
t1781	STY1176	STM1139	csgG	assembly/transport component in curli production
t2457	STY0444	STM0406	yajC	putative membrane protein
t2787	STY3008	STM2885	sipB	pathogenicity island 1 effector protein
t2558	STY0336	STM0301	safC	outer-membrane fimbrial usher protein
t3352	STY3614	STM3945		possible exported protein
t1870	STY1070			putative bacteriophage protein

Characterization of Anti-*Salmonella enterica* Serotype Typhi Antibody Responses in Bacteremic Bangladeshi Patients by an Immunoaffinity Proteomics-Based Technology^{7,†}

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Salmonella enterica serotype Typhi is the cause of typhoid fever and available typhoid vaccines provide 50 to 90% protection for 2 to 5 years. Assays to identify individuals with typhoid fever lack sensitivity and/or specificity.

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IPT TO CHARACTERIZE *S. TYPHI* IMMUNOPROTEOME 1193

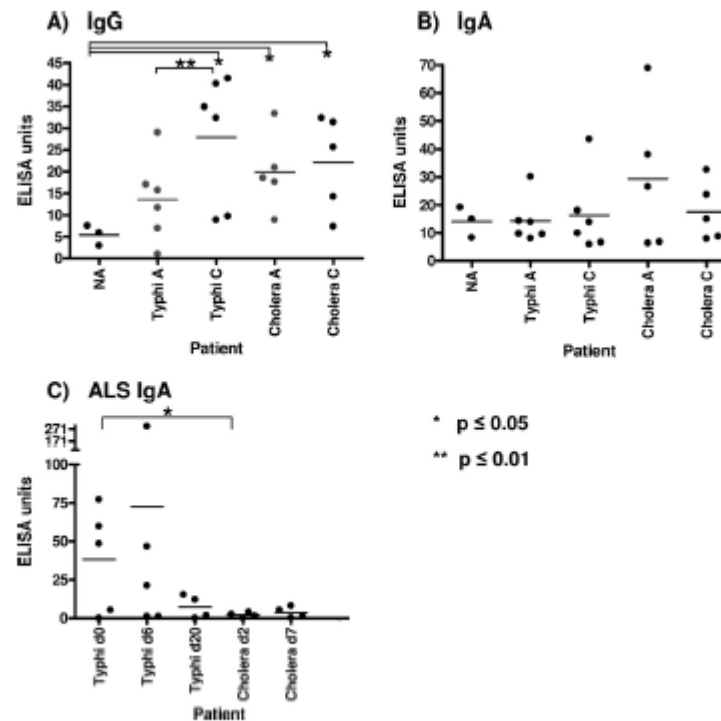


FIG. 1. Characterization of anti-HlyE immune responses. Anti-HlyE IgG (A) and IgA (B) responses in the plasma of North American volunteers (NA) and acute-phase (day 0) and convalescent-phase (day 20) plasma of patients infected with *S. Typhi* or *V. cholerae* (control). (C) Anti-HlyE IgA ALS responses in day 0, day 6, and day 20 samples of patients infected with *S. Typhi* and in day 2 and day 7 samples of patients infected with *V. cholerae* (control).

Summary

- We have identified a sub-set of proteins in MP, that can be advanced for next generation TPTest based detection systems.
- We have found that TPTest fluid is both sensitive and specific for detecting acute stage infection, even in edemic zones.
- These findings probably also applicable to other infections.

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