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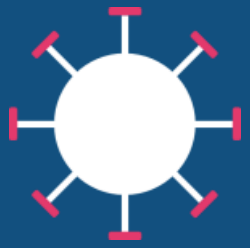


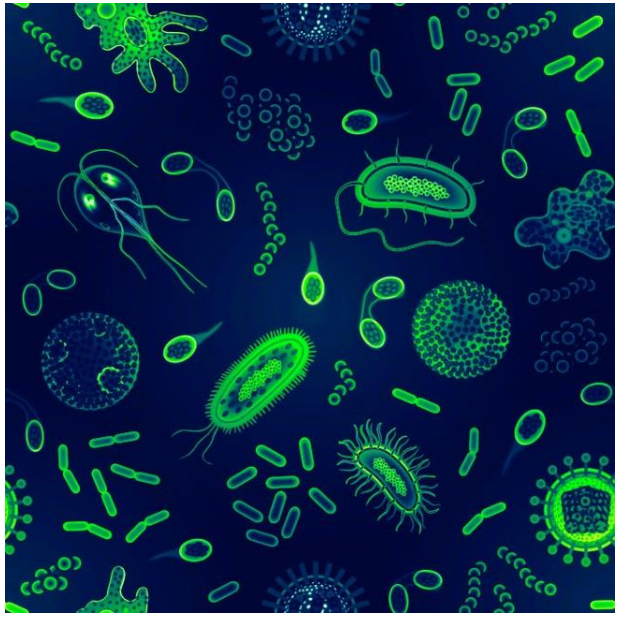
Case Study: IgG and IgA Antibody Profiling with the PEPperCHIP® Infectious Disease Epitope Microarray

Serum Signature Analysis

PEPperPRINT GmbH
Heidelberg, 04/2015

Introduction

The diagnosis of infectious diseases is often hampered by the display of identical symptoms caused by different pathogens. To make a definitive diagnosis, individual assays are needed for each suspected pathogen leading to greater costs and increased time consumption. A better solution would be a highly multiplexed assay that enables the analysis of antibody responses against variety of pathogens at once. Therefore, we developed the PEPperCHIP® Infectious Disease Epitope Microarray based on 3,857 B-cell epitopes associated with 191 different pathogens to enable the multiplexed screening of IgG, IgM and IgA antibody responses against more than 100 infectious diseases in a single assay.



The pathogen epitopes were selected from the Immune Epitope Database (www.iedb.org) and correlate with a variety of frequent and less common infections such as various hemorrhagic fevers, Lyme disease, measles, Chagas, RSV, tuberculosis, candidiasis, malaria, hepatitis, influenza, and many more.

The PEPperCHIP® Infectious Disease Epitope Microarray enables the in-depth serum profiling of patients with unknown infections against 191 different pathogens at once to e.g. detect hidden secondary infections, or to investigate a correlation of autoimmune diseases with B-cell responses raised by infections. Thereby, this new peptide microarray supports clinicians making treatment decisions and risk assessments, and provides a valuable tool for autoimmune and infection disease research.

In this report, we describe the detailed analysis of IgG and IgA antibody responses of the serum of a 56 year old female diabetes mellitus type 1 patient with the PEPperCHIP® Infectious Disease Epitope Microarray. That way we identified a number of antibody responses against various viral and bacterial antigens including previously unknown Helicobacter pylori and Toxoplasma gondii infections. The report exemplifies the data set that is provided by PEPperPRINT in line with a typical PEPperMAP® Service. It includes the scientific report as PowerPoint file as well as all microarray data and intensity calculations in Excel format.

Material and Methods

Microarray Content:	The PEPperCHIP® Infectious Disease Epitope Microarray covers 3,857 linear B-cell epitopes associated with 191 different pathogens of more than 100 infectious diseases from the Immune Epitope Database (http://www.iedb.org/). Database epitopes of more than 16 aa were translated into overlapping 15 aa peptides. The PEPperCHIP® Infectious Disease Epitope Microarray contains 4,336 different peptides printed in duplicate as well as additional Flag (DYKDDDDKAS) and HA (YPYDVPDYAG) control peptides (152 spots each control).
Sample:	Human Serum 181641 (56 year old female patient with Diabetes mellitus Type 1)
Washing Buffer:	PBS, pH 7.4 with 0.05% Tween 20 (3x1 min after each assay)
Blocking Buffer:	Rockland blocking buffer MB-070 (30 min before the first assay)
Incubation Buffer:	PBS, pH 7.4 with 0.05% Tween 20 and 10% Rockland blocking buffer
Assay Conditions:	Serum dilution of 1:250 in incubation buffer; incubation for 16 h at 4°C and shaking at 140 rpm
Secondary Antibodies:	Goat anti-human IgG (Fc) conj. DyLight680 (1:1000), goat anti-human IgA (alpha) conj. DyLight800 (1:1000); 45 min staining in incubation buffer at RT
Control Antibody:	Monoclonal anti-HA (12CA5)-DyLight800 (1:1000); 45 min staining in incubation buffer at RT
Scanner:	LI-COR Odyssey Imaging System; scanning offset 0.8 mm, resolution 21 µm, scanning intensities of 6/7 (red = 700 nm/green = 800 nm)
Microarray Data:	Microarray Data Secondary & Control Antibodies.xlsx, Microarray Data Serum 181641.xlsx, Microarray Data Serum 181641 (Hit Epitope Mapping).xlsx
Microarray Identifier:	000784_03, 000784_09 (PEPperCHIP® Infectious Disease Epitope Microarray), 000946_04 (Epitope Mapping Peptide Microarray)

Experimental and Data Analysis

After 15 min pre-swelling in standard buffer and 30 min in blocking buffer, a PEPperCHIP® Infectious Disease Epitope Microarray was initially incubated with the secondary antibodies goat anti-human IgG (Fc) conj. DyLight680 and goat anti-human IgA (alpha) DyLight800 at dilutions of 1:1000 in the presence of the control antibody monoclonal anti-HA (12CA5)-DyLight800 (1:1000) for 45 min at room temperature to analyze background interactions with the infectious disease epitopes. Subsequent incubation of the PEPperCHIP® Infectious Disease Epitope Microarray with human serum sample 181641 at a dilution of 1:250 in incubation buffer was followed by staining with the secondary antibodies in the presence of the monoclonal anti-HA (12CA5)-DyLight800 control antibody and read-out at scanning intensities of 6/7 (red/green). HA control peptides were simultaneously stained as internal quality control to confirm the assay quality and to facilitate grid alignment for data quantification.

Quantification of spot intensities and peptide annotation were based on 16-bit gray scale tiff files that exhibit a higher dynamic range than 24-bit colorized tiff files; microarray image analysis was done with PepSlide® Analyzer and summarized in the Excel files “Microarray Data Secondary & Control Antibodies.xlsx” and “Microarray Data Serum 181641.xlsx”. A software algorithm breaks down fluorescence intensities of each spot into raw, foreground and background signal (see “Raw Data” tabs), and calculates the standard deviation of median foreground intensities (see “Microarray Data Summary” tabs). Spots with a deviation of 40% were zeroed to yield corrected averaged foreground intensities. Based on corrected averaged median foreground intensities, intensity maps were generated and binders in the peptide maps highlighted by an intensity color code with red (IgG, 700 nm) and green (IgA, 800 nm) for high and white for low spot intensities.

To identify the top IgG and IgA responses, the averaged and corrected intensity values were sorted by decreasing intensities (see “Top Responses” tabs). We further plotted averaged spot intensities of the pre-staining and the assay with human serum sample 181641 against the microarray content from left on top to right on bottom of the chip to visualize overall spot intensities and signal-to-noise ratios (see “Intensity Plot” tabs). The intensity plots were correlated with peptide and intensity maps as well as with visual inspection of the microarray scan to identify infectious disease epitopes that showed an IgG or IgA response with human serum sample 181641.

Pre-Staining, Secondary Antibodies

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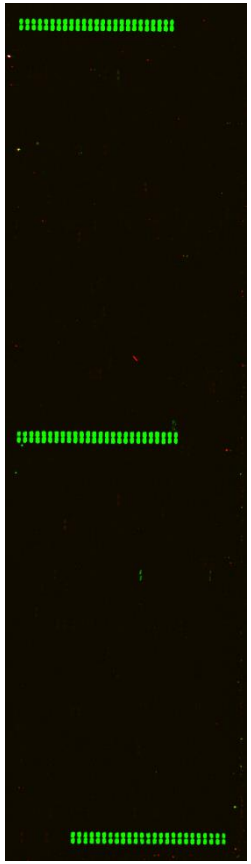
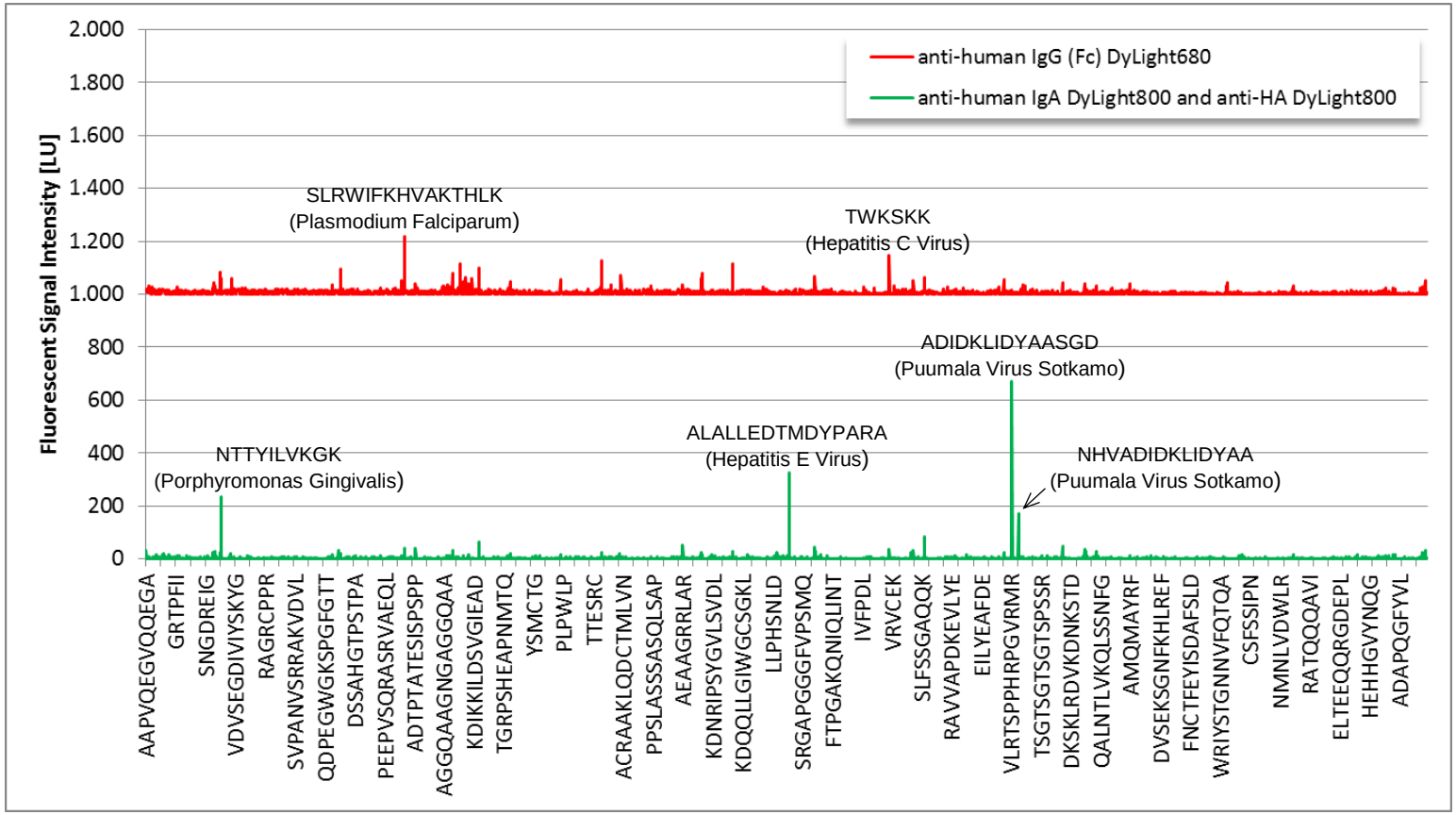


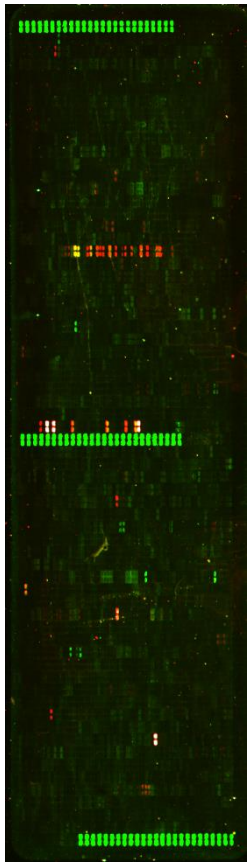
Goat anti-human IgG (Fc) conj. DyLight680 (1:1000), goat anti-human IgA (alpha) DyLight800 (1:1000) and monoclonal anti-HA (12CA5)-DyLight800 antibody (1:1000)



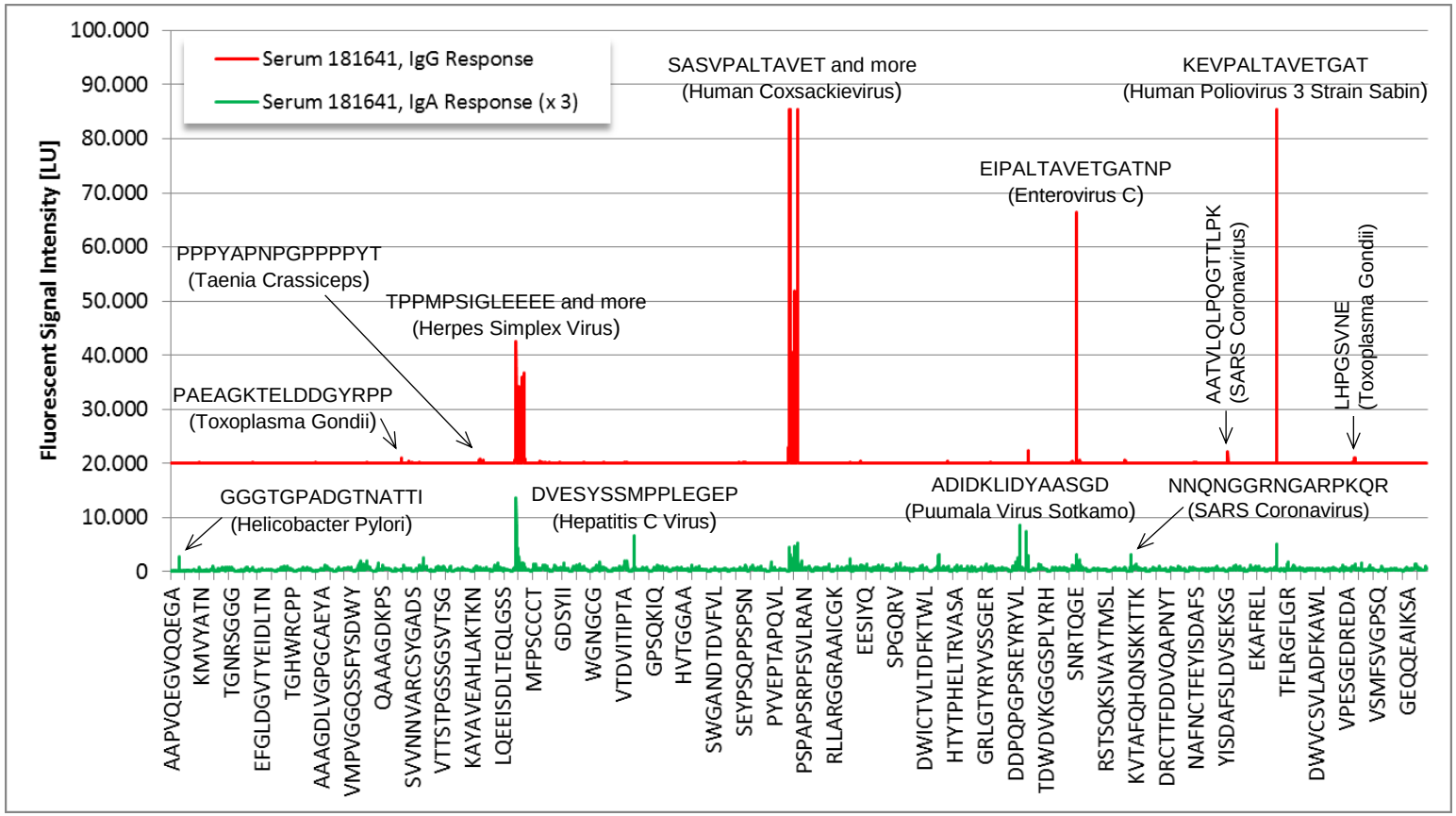
Initially, a PEPperCHIP® Infectious Disease Epitope Microarray was incubated with secondary and control antibodies to investigate background interactions with infectious disease epitopes; the microarray scan showed a clear response against the control peptides on top, in the middle and on bottom of the microarray (left). Moreover, we observed few low intensity background interactions of secondary and control antibodies that were annotated in the intensity plots next to the corresponding signal; for a better data overview, the intensity plot of the red channel at 700 nm was leveled. The background interactions were either attributed to cross-reactions of the monoclonal anti-HA control antibody, or to non-specific ionic interactions with basic peptides like TWKSKK or SLRWIFKHVAKTHLK.

Human Serum 181641 – Scan & Plot

000784_09



Human Serum 181641 (1:250)



Human serum 181641 was assayed at a dilution of 1:250 followed by staining with secondary and control antibodies; the microarray scan showed a complex response pattern with the control peptides on top, in the middle and on bottom of the microarray (left). We observed some common IgG and IgA responses (yellow spots), but also clearly differential interactions for either IgG (red) or IgA antibodies (green). The IgG and IgA response patterns were visualized by intensity plots (right); for a better data overview, the plot of the IgG response was leveled, and the spot intensities of the IgA response were tripled. The main IgG and IgA responses were annotated next to the corresponding signal in the intensity plots.

Human Serum 181641 – IgG Responses

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Peptide	Serum 181641, IgA Response	Serum 181641, IgG Response	Epitope ID	Source Molecule Accession	Source Molecule Name	Source Organism ID	Source Organism Name
HSKEIPALTAVETGA	1.554,0	65.357,0	88446	25121843	Coat Protein Vp1	12081	Human Poliovirus 1 Mahoney
KEVPALTAVETGAT	1.679,0	65.357,0	30661	217315555	Vp1	270338	Human Poliovirus 3 Strain Sabin
SASVPALTAVET	1.535,0	65.357,0	135990	6730243	Chain 1, The Crystal Structure Of Coxsack	12067	Human Coxsackievirus A9
VPALTAVETGHT	1.046,0	65.357,0	136011	6730243	Chain 1, The Crystal Structure Of Coxsack	12067	Human Coxsackievirus A9
EIPALTAVETGATNP	1.067,5	46.504,5	81565	25121843	Coat Protein Vp1	138950	Enterovirus C
PALTAAETG	1.548,5	31.827,5	120682	61032	Polyprotein Cb4	12073	Human Coxsackievirus B4
PALTAVETGATNPL	533,0	27.113,5	80446	25121843	Coat Protein Vp1	12081	Human Poliovirus 1 Mahoney
EEEEGAGDGEHLE	4.577,5	22.570,0	11579	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
IPALTAAETGHTSQV	866,5	20.700,5	83789	31247842	Polyprotein	12071	Human Coxsackievirus B1
GPTHSKEIPALTAVE	1.773,0	20.420,0	83234	25121843	Coat Protein Vp1	12081	Human Poliovirus 1 Mahoney
ETIYNTTLKY	456,5	16.753,0	14435	138192	Glycoprotein B Precursor	10359	Human Herpesvirus 5
TPPMPSIGLEEEE	574,0	16.036,0	65691	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
MPSIGLEEEEEEE	936,0	14.231,5	42322	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
TSKGRPLVPTPQH	147,0	12.352,0	66281	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
EGAGDGEHLEGGD	2.702,5	9.979,5	12118	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
IGLEEEEEEEGAG	471,5	5.122,5	26265	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
PAFPLAEDVEKDK	170,5	4.522,5	46796	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
GDGEHLEGGDGTR	1.440,0	4.094,5	18985	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
LPQSPGPAFPLAE	190,5	3.741,0	38660	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
PEEFEGAGDGEPPED	530,5	3.662,5	47266	9629334	Envelope Glycoprotein G	10310	Human Herpesvirus 2
EKDKPNRPVVPSP	174,0	3.280,0	12647	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
LTAVETGHTSQV	60,5	2.989,5	135958	6730243	Chain 1, The Crystal Structure Of Coxsack	12067	Human Coxsackievirus A9
KPNRPVVPSPDPN	132,0	2.600,0	32812	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
RGLAQALIDQ	972,0	2.431,0	53905	127920	Nucleoprotein	39002	Puumala Virus Sotkamo/V-2969/81
PLAEDVEKDKPNR	189,5	2.283,0	48271	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
AATVLQLPQGTTLPK	103,5	2.121,5	475	30027624	N Protein	228407	Sars Coronavirus Bj01
RDTLPQSPGPAFP	272,5	1.930,5	53429	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
PAEAGKTELDDGYRPP	0,0	1.015,0	167983	2498423	Dense Granule Protein 4 Precursor	5811	Toxoplasma Gondii
LHPGSVNE	204,0	993,5	167617	237845283	Granule Antigen Protein Gra6	508771	Toxoplasma Gondii Me49
LHPGSVNEFD	375,0	956,5	167618	237845283	Granule Antigen Protein Gra6	508771	Toxoplasma Gondii Me49
PPPYAPNPGPPPPYT	277,0	870,0	77968	968978	Protective Recombinant Antigen	6207	Taenia Crassiceps
PPPGRRPFFHPVGE	268,0	864,5	48852	119110	Epstein-Barr Nuclear Antigen 1	10377	Human Herpesvirus 4 (Strain B95-8)
EDVEKDKPNRPVV	143,0	852,5	11457	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
LHPGSVNEFDF	478,0	784,5	167619	237845283	Granule Antigen Protein Gra6	508771	Toxoplasma Gondii Me49
DGTRDTLPQSPGP	284,0	620,5	8503	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
GGDEYVTKGEVVETD	199,0	611,5	138910	3599467	Abundant Larval Transcript-2 Protein	6293	Wuchereria Bancrofti
QLPQGTTLPKGFYAE	256,5	595,0	51483	30173007	Nucleoprotein	227859	Sars Coronavirus
PDPNNSPARPETS	94,0	592,5	47159	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
EDPSVEEEEIAPPLPP	749,0	585,0	119570	88658350	Ankyrin Repeat-Containing Protein	205920	Ehrlichia Chaffeensis Str. Arkansas
MPPYPTGGPPPV	254,0	580,0	42308	968978	Protective Recombinant Antigen	6207	Taenia Crassiceps

The table summarizes the top 40 IgG responses of human serum 181641 at a dilution of 1:250 sorted by decreasing spot intensities after removal of spots with a spot-to-spot deviation of >40%. We observed very strong responses against a number of very similar epitopes of human poliovirus/coxsackievirus, but also clear interactions with multiple herpes simplex virus epitopes and against human herpesvirus 5. Multiple IgG responses were also identified for epitopes of Toxoplasma gondii, Taenia crassiceps and surprisingly SARS coronavirus, albeit on a significantly lower intensity level.

Human Serum 181641 – IgA Responses

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Peptide	Serum 181641, IgA Response	Serum 181641, IgG Response	Epitope ID	Source Molecule Accession	Source Molecule Name	Source Organism ID	Source Organism Name
EEEEGAGDGEHLE	4.577,5	22.570,0	11579	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
ADIDKLIDYAASGD	2.849,0	49,0	677	127920	Nucleoprotein	39002	Puumala Virus Sotkamo/V-2969/81
EGAGDGEHLEGGD	2.702,5	9.979,5	12118	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
NHVADIDKLIDYAA	2.476,5	35,5	44172	127920	Nucleoprotein	39002	Puumala Virus Sotkamo/V-2969/81
DVESYSSMPPLEGEP	2.256,0	51,0	8877	130469	Genome Polyprotein	11103	Hepatitis C Virus
GPTHSKEIPALTAVE	1.773,0	20.420,0	83234	25121843	Coat Protein Vp1	12081	Human Poliovirus 1 Mahoney
KEVPALTAVETGAT	1.679,0	65.357,0	30661	217315555	Vp1	270338	Human Poliovirus 3 Strain Sabin
HSKEIPALTAVETGA	1.554,0	65.357,0	88446	25121843	Coat Protein Vp1	12081	Human Poliovirus 1 Mahoney
PALTAAETG	1.548,5	31.827,5	120682	61032	Polyprotein Cb4	12073	Human Coxsackievirus B4
SASVPALTAVET	1.535,0	65.357,0	135990	6730243	Chain 1, The Crystal Structure Of Coxsack	12067	Human Coxsackievirus A9
GDGEHLEGGDGTR	1.440,0	4.094,5	18985	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
EIPALTAVETGATNP	1.067,5	46.504,5	81565	25121843	Coat Protein Vp1	138950	Enterovirus C
VPALTAVETGHT	1.046,0	65.357,0	136011	6730243	Chain 1, The Crystal Structure Of Coxsack	12067	Human Coxsackievirus A9
NNQNGGRNGARPKQR	1.035,5	51,0	61559	30173007	Nucleoprotein	227859	Sars Coronavirus
SYSSMPPLEGEPGDP	1.031,0	22,0	176654	12831193	Polyprotein	31647	Hepatitis C Virus Subtype 1B
SSMPPLEGEPGDPDL	979,0	39,0	176654	12831193	Polyprotein	31647	Hepatitis C Virus Subtype 1B
RGLAQALIDQ	972,0	2.431,0	53905	127920	Nucleoprotein	39002	Puumala Virus Sotkamo/V-2969/81
MPSIGLEEEEEEE	936,0	14.231,5	42322	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
GGGTGPADGTNATTI	904,5	17,0	136315	57014162	Urease Subunit Beta	210	Helicobacter Pylori
IPALTAAETGHTSQV	866,5	20.700,5	83789	31247842	Polyprotein	12071	Human Coxsackievirus B1
EENVEHDAEENVEHDA	857,0	104,0	11821	410748	Ring-Infested Erythrocyte Surface Antige	5833	Plasmodium Falciparum
VEEQEQEQEEQEEQE	847,0	147,5	68167	139472804	Lana	37296	Human Herpesvirus 8
EVLYREFDE	803,5	173,5	14822	169244841	Polyprotein [Hepatitis C Virus Subtype 1	31646	Hepatitis C Virus Subtype 1A
EDPSVEEEEIAPPLPP	749,0	585,0	119570	88658350	Ankyrin Repeat-Containing Protein	205920	Ehrlichia Chaffeensis Str. Arkansas
EQEQEQEEQEEQELE	746,0	129,0	68167	139472804	Lana	37296	Human Herpesvirus 8
EQEQEQEEQELEEVE	743,5	128,0	68168	139472804	Lana	37296	Human Herpesvirus 8
EEEEEEEGAGDGE	713,5	181,5	11576	9629445	Envelope Glycoprotein G	10299	Herpes Simplex Virus (Type 1 / Strain 17)
VEEQEQEQEEQELEE	705,5	130,0	68168	139472804	Lana	37296	Human Herpesvirus 8
KEFED	679,0	143,0	30370	994798	Heat Shock Protein 90	5476	Candida Albicans
PHSALALLEDTMDYP	678,0	48,0	2045	10336835	Structural Protein	12461	Hepatitis E Virus
VLYREFDE	671,0	165,0	69899	130455	Genome Polyprotein	11103	Hepatitis C Virus
LYREFD	658,0	157,0	40922	130455	Genome Polyprotein	11103	Hepatitis C Virus
EDDDMGFGLFD	643,5	176,0	119823	10638	Ribosomal Protein P-Jl5	5693	Trypanosoma Cruzi
IGQMFE	628,0	110,5	26307	160336975	Envelope Protein	11064	Dengue Virus 2 Jamaica/1409/1983
YAAQNRRGLDLLFWE	623,0	85,5	73327	119464	Envelope Glycoprotein Gp62 Precursor	11908	Human T-Lymphotropic Virus 1
YVGNYDFWY	620,5	117,0	76285	49483582	Abc Transporter Atp-Binding Protein	282458	Staphylococcus Aureus Subsp. Aureus Mrsa252
QEEQEQQEEQEQELE	598,0	113,0	50578	139472804	Lana	37296	Human Herpesvirus 8
REVLYREFDEME	597,5	131,5	53667	130455	Genome Polyprotein	11103	Hepatitis C Virus
EENVEHDAEENVEENV	581,0	76,0	11820	410748	Ring-Infested Erythrocyte Surface Antige	5833	Plasmodium Falciparum
AESEE	576,0	110,0	1157	20141794	Protein Transport Protein Sec23B	9606	Homo Sapiens

The table summarizes the top 40 IgA responses of human serum 181641 at a dilution of 1:250 sorted by decreasing spot intensities after removal of spots with a spot-to-spot deviation of >40%. Background interactions of the control and/or secondary antibodies were highlighted in light blue. Besides main interactions against epitopes of human poliovirus/coxsackievirus and herpes simplex virus, we also identified multiple IgA responses against epitopes of hepatitis C virus, Helicobacter pylori and surprisingly also against SARS coronavirus. A number of low intensity IgA responses were based on acidic peptides and likely resulted from less specific cross-reactions.

Discussion

Approach: To demonstrate the potential and the use of the PEPperCHIP® Infectious Disease Epitope Microarray, we incubated one of the microarrays with human test serum 181641 at a dilution of 1:250 in incubation buffer. After incubation, staining with the secondary antibodies goat anti-human IgG (Fc) conj. DyLight680 and goat anti-human IgA (alpha) DyLight800 together with the monoclonal anti-HA (12CA5)-DyLight800 control antibody was followed by read-out with a LI-COR Odyssey Imaging System. Quantification of spot intensities and peptide annotation were done with PepSlide® Analyzer.

IgG Response: Human serum 181641 showed a strong IgG response against a number of peptides at high spot intensities and signal to noise ratios. The strongest interactions were directed against epitopes of human coxsackievirus A9 (VPALTAVETGHT, SASVPALTAVET, EAIPALTAVETGHTSQV) and human poliovirus (HSKEIPALTAVETGA, KEVPALTAVETGAT). Due to the pronounced sequence similarity of the coxsackievirus and poliovirus epitopes, we assume that the main response was actually directed against epitope KEVPALTAVETGAT of human poliovirus 3 strain Sabin and raised by a polio immunization, whereas the coxsackievirus responses resulted from cross-reactions of the corresponding antibody. Other strong IgG responses were directed against various epitopes of herpes simplex virus and against a single epitope of human herpesvirus 5; both are common infections with a very high rate of infestation.

Less strong IgG responses were observed against multiple epitopes of Toxoplasma gondii, a parasitic protozoan that causes toxoplasmosis, and Taenia crassiceps, a tapeworm that can be found in immunocompromised patients. Surprisingly, we also identified a number of responses against SARS coronavirus epitopes. With decreasing spot intensities below 1,000 fluorescence units, a number of additional less likely IgG responses against single epitopes of e.g. Wuchereria bancrofti or Ehrlichia chaffeensis appeared. Since such weak and singular interactions can be caused by non-specific binding events or antibody cross-reactions, we did not take them into account for further validation and analysis.

IgA Response: Compared to the IgG response, the IgA response was significantly less strong and clear. The main interactions were again found for various epitopes of human poliovirus/coxsackievirus and herpes simplex virus, what was in accordance with the IgG response.

Discussion

In addition, we also identified IgA responses against epitopes of hepatitis C virus, Helicobacter pylori and surprisingly again against SARS coronavirus. Some additional very low intensity IgA interactions were mainly based on acidic peptides and likely resulted from less specific cross-reactions. A response against SARS coronavirus was also found in the IgG profile and needs to be validated, since a corresponding infection was regarded as unlikely. The same applied to the IgA responses against epitopes of hepatitis C virus without any accompanying IgG response; this may rather hint at a cross-reaction with other IgA antibodies, but not at an actual HCV infection. In contrast to this, an IgA response against Helicobacter pylori appeared to be reasonable due to its high prevalence particularly in the gastrointestinal tract.

Diagnostic Test: To validate the HCV and Helicobacter pylori, 800 µl of human test serum 181641 were provided to a diagnostic lab; the standard tests revealed that the serum was actually HCV negative, what was expected from the missing IgG response. However, the IgA response against Helicobacter pylori could be validated with 58 U/ml total antibody.

Additional Epitope Mapping: To further validate the IgG responses against Toxoplasma gondii and Taenia crassiceps as well as the observed IgG and IgA interactions with SARS coronavirus epitopes, we decided to perform an additional multiplexed epitope mapping against the corresponding antigens of these pathogens. The antigens were selected according to the observed epitope responses, and the full antigen sequences translated into overlapping peptides printed on a second peptide microarray. Instead of using 400-500 µl serum for a single diagnostic test, such a multiplexed epitope mapping enables the identification of conserved epitopes and mono- or polyclonal responses against one or more antigens with less than 10 µl serum.

The epitope mapping peptide microarray was based on nucleocapsid protein (AAY28827.2, Puumala virus), nucleocapsid protein (ACZ72205.1, SARS coronavirus), dense granule protein GRA4 (KFG40958.1, Toxoplasma gondii p89), granule antigen protein (CAG25735.1, Toxoplasma gondii) and protective recombinant antigen (AAB02180.1, Taenia crassiceps). The antigens were translated into 15 aa peptides with a maximum peptide-peptide overlap of 14 amino acids resulting in an epitope mapping peptide microarray with 1,357 different peptides printed in duplicate including again Flag and HA control peptides (110 spots each control).

Serum 181641 – Hit Epitope Mapping

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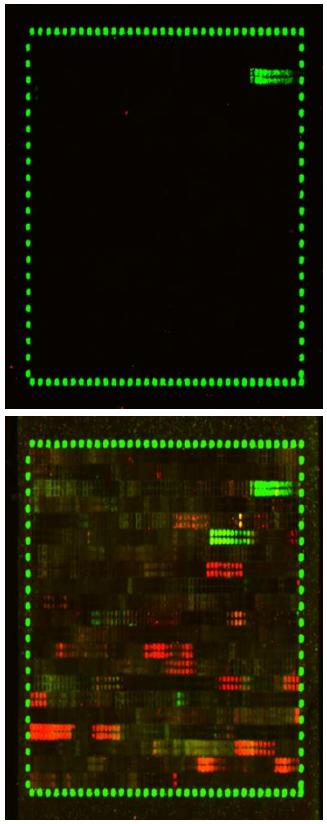
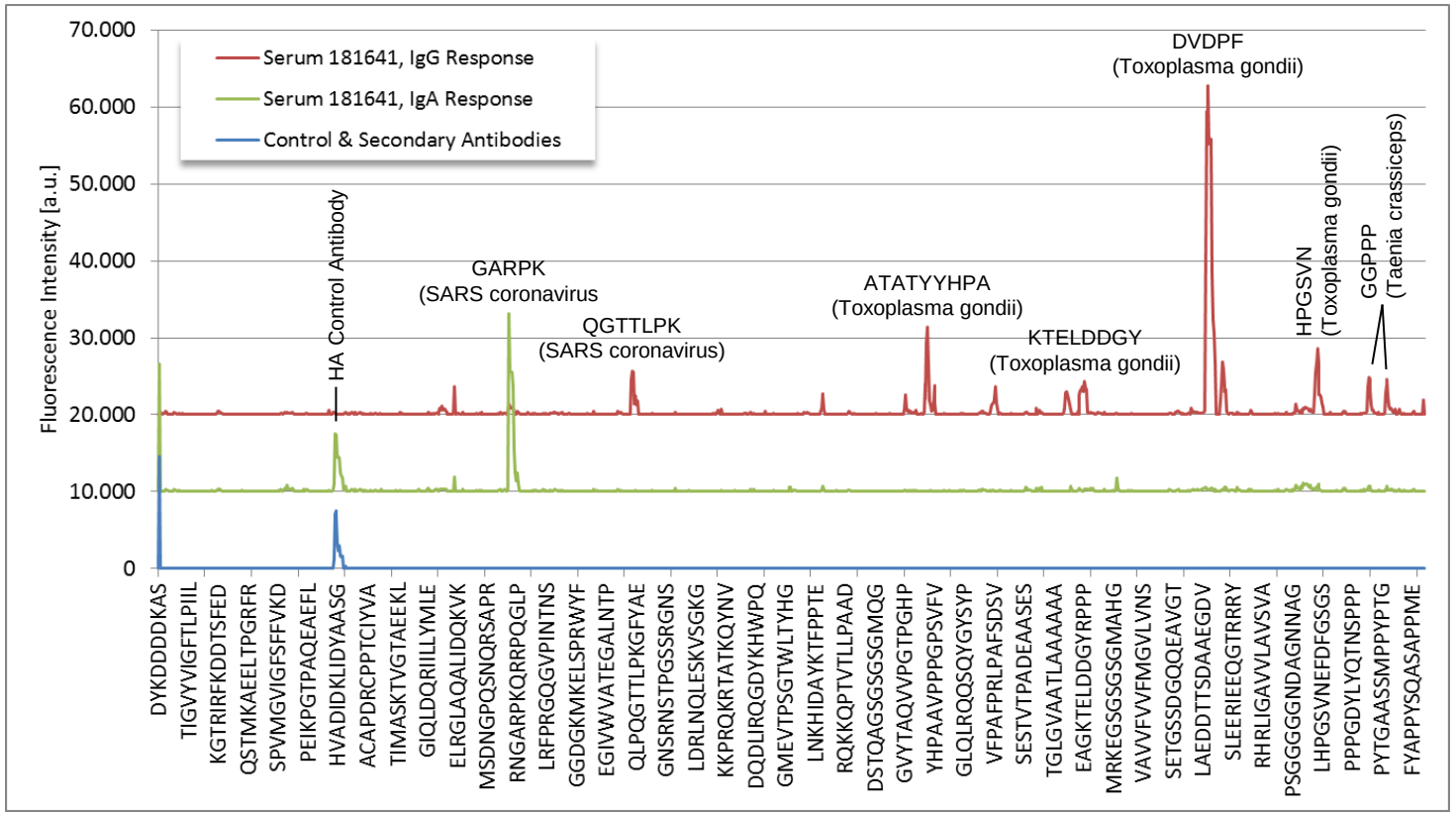


Pre-Staining and Human Serum 181641 (1:250), Scanning Intensities of 6/7 (IgG and IgA)



The epitope mapping peptide microarray was initially incubated with secondary and control antibodies followed by read-out at scanning intensities of 6/7 (red/green, left on top); we observed a clear and well-defined epitope-like spot pattern formed by a row of adjacent peptides against peptides with the consensus motif DKLIDYA, likely due to a cross-reaction with the anti-HA (12CA5)-DyLight800 antibody. Incubation of another epitope mapping peptide microarray with human serum 181641 at a dilution of 1:250 followed by staining with secondary and control antibodies revealed strongly polyclonal IgG and IgA responses against virtually all antigens covered by the microarray (left on bottom). The intensity plots on right highlighted the corresponding IgG and IgA response patterns in comparison with the pre-staining with control and secondary antibodies; the main epitopes were annotated next to the corresponding signal.

Discussion and Conclusion

Approach: To validate the IgG and IgA responses against epitopes of Toxoplasma gondii, Taenia crassiceps and SARS coronavirus as well as the supposed response of the monoclonal anti-HA (12CA5)-DyLight800 antibody against Puumala virus epitopes, we generated an epitope mapping peptide microarray with 5 pathogen antigens translated into overlapping peptides with maximum epitope coverage. The resulting epitope mapping peptide microarray was incubated with human serum 181641 at a dilution of 1:250 (serum consumption ~3 µl) followed by staining with secondary and control antibodies.

Pre-Staining: In the pre-staining with secondary and control antibodies, we already observed a clear epitope-like spot pattern formed by a row of adjacent peptides in the green channel (goat anti-human IgA (alpha) conj. DyLight800 and monoclonal anti-HA (12CA5)-DyLight800). The corresponding spot pattern was assigned to peptides with the consensus motif DKLIDYA that exhibited a pronounced similarity with the wild type HA control peptide YPYDVPDYAG. Therefore we attributed this response against Puumala virus to the anti-HA control antibody, and thereby confirmed the assumed cross-reaction of the PEPperCHIP® Infectious Disease Epitope Microarray.

IgG and IgA Responses: The IgG profile highlighted a strong and clear epitope-like spot pattern against SARS coronavirus based on peptides with the consensus motif QGTTLPK, a very strong and polyclonal response against Toxoplasma gondii with a variety of clear epitopes as well as two epitope-like spot patterns against Taenia crassiceps. While the IgG response against Toxoplasma gondii was validated by the highly polyclonal character, the two Taenia crassiceps spot patterns exhibited the same short consensus motif GGPPP. Such a short epitope can cause cross-reactions with other antibody species and does not unambiguously underline a Taenia crassiceps infection that further requires immunocompromised patients. Taking the missing polyclonality against protective recombinant antigen of Taenia crassiceps into account, it is reasonable to assume that the observed IgG response of the PEPperCHIP® Infectious Disease Epitope Microarray was in fact caused by such cross-reaction, and not by an actual Taenia crassiceps infection.

In addition to the IgG response against SARS coronavirus epitope QGTTLPK, we further identified a clear IgA response against peptides with the consensus motif GARPK, what validated the interactions of the PEPperCHIP® Infectious Disease Epitope Microarray.

Discussion and Conclusion

Since an actual SARS coronavirus infection has to be regarded as very unlikely, we run a blast search of both epitopes QGTTLPK and GARPK in protein databases to identify other pathogens with similar epitopes.[1] Interestingly, we found two very similar sequences QGTTLP and GARPK in Escherichia coli. Similar to Helicobacter pylori, Escherichia coli has a high prevalence and is frequently found in the gastrointestinal tract, what would be in accordance with the clear and strong IgA response. Therefore we concluded that the presumable SARS coronavirus responses of the PEPperCHIP® Infectious Disease Epitope Microarray were likely raised by Escherichia coli.

Summary: We used the new PEPperCHIP® Infectious Disease Epitope Microarray based on 3,857 B-cell epitopes of the Immune Epitope Database associated with 191 different pathogens to screen for IgG and IgA antibody profiles in human test serum 181641 of a 56 year old female patient with Diabetes mellitus Type 1. We identified very strong IgG responses against human poliovirus/coxsackievirus epitopes as well as against herpes simplex virus and human herpesvirus 5 epitopes. Weaker IgG interactions were found for epitopes of Toxoplasma gondii, Taenia crassiceps and surprisingly SARS coronavirus. The two latter, however, were possibly based on cross-reactions with other antibody species; particularly the apparent SARS coronavirus was likely based on a more reasonable Escherichia coli infection, as validated by a subsequent high resolution epitope mapping and a protein blast search of the conserved core epitope.

The main IgA responses of the PEPperCHIP® Infectious Disease Epitope Microarray were also directed against human poliovirus/coxsackievirus and herpes simplex virus epitopes. Moreover, we found a weak IgA response against Helicobacter pylori that was previously unknown and validated by a standard diagnostic test, albeit with a high sample consumption of ~400 µl. As suspected, the IgA responses against HCV epitopes without any accompanying IgG response were false-positive hits due to e.g. cross-reactions with other antibodies or non-specific binding events. The surprising IgA response against SARS coronavirus could actually be confirmed with the epitope mapping peptide microarray, but was most likely caused by a Escherichia coli infection with an identical conserved core motif.

[1] NCBI Protein Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi)

Discussion and Conclusion

Due to the high serum need for standard diagnostic tests, some of the less expected IgG and IgA hits were investigated in a subsequent multiplexed epitope mapping of 5 different pathogen antigens with a total sample consumption of only ~3 µl. It turned out that all responses of the PEPperCHIP® Infectious Disease Epitope Microarray were actually based on specific interactions, albeit in part against other pathogens with similar epitopes, as shown for the apparent SARS coronavirus infection. The supposed cross-reaction of control and/or secondary antibodies against Puumala virus could be assigned to the anti-HA control antibody, and the rather weak response against Toxoplasma gondii validated by a strong and polyclonal IgG response on the epitope mapping peptide microarray.

Another option for epitope validation, an in-depth epitope analysis and particularly the identification of conserved and variable amino acid positions is given by a PEPperMAP® Full Substitution Scan of all amino acid positions with the 20 main amino acids (see also application note "PEPperMAP® Full Substitution Scan of SMN Epitope").

With higher sample numbers and particularly a comparison of patient and control groups, the new PEPperCHIP® Infectious Disease Epitope Microarray can be used to identify differential serum biomarkers with a prognostic value, for a correlation of autoimmune diseases with B-cell responses raised by infections, or to differentiate between infected and vaccinated patients in vaccine research and development.

It should also be pointed out that PEPperCHIP® Peptide Microarrays are fully compatible with standard ELISA tests and immunoassays. Moreover, the peptide and epitope content of PEPperCHIP® Peptide Microarrays can be easily adjusted with respect to custom antigens, epitopes or organisms in a uniquely flexible manner.

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