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Serum Epitope Repertoire Analysis (SERA) Enables Serologic Immune Monitoring

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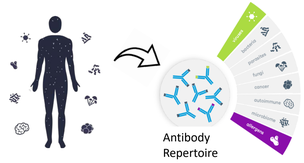
Abstract

The human immune system is a dynamic record of recent and historic environmental exposures throughout a person's life. The ability to profile and monitor the changing immune landscape has broad clinical relevance on an individual level and within populations, from driving healthcare decisions, to biomarker development, to revealing cryptic disease relationships. The timing of infection is also important. For example, distinguishing acute infection or reactivation from previous or latent infection during pregnancy, aging and in immunocompromised settings is critical for guiding healthcare decisions. Conventional serology often relies upon measurements of IgM antibodies to identify acute infections. Because IgM is less specific, it can require use of more complex testing algorithms to confirm acute infection. Current tests are also limited in that they only provide information about suspected infections, and unsuspected diseases go undiagnosed. Development of new multiplex tools, including antigen and peptide arrays, has improved upon the "one test-one disease" diagnostic paradigm, but are limited by the number of total antigens or organisms that can be printed, the knowledge of which antigens or epitopes are important in the immune response and the need to select *a priori* which diseases will be included.

Serimmune has developed a Serum Epitope Repertoire Analysis platform (SERA) that allows quantitative testing of an unlimited number of diseases in a single, universal assay. Using bacterial display random peptide libraries and next-generation sequencing to profile antibody-binding peptides, we have built a database of thousands of epitope repertoires from healthy individuals, and those with infections, autoimmune disease, and cancer. Leveraging this database, we have discovered panels of epitope motifs for 28 distinct infectious diseases and motifs that are conserved across all cohorts (20-90%) and map to common human exposures. Once processed, an epitope repertoire may be queried for any number of diseases simultaneously. Repertoires can also be analyzed retrospectively as new panels are developed. Here we describe the discovery platform and present the data on the epitope motif panels for common diseases including CMV, EBV, Toxoplasmosis and HSV-2, epitope mapping to known and novel antigens, and seroprevalence within our database as compared with published seroprevalence. We also address the broader implications of the technology in monitoring human immunity to diverse infectious agents throughout life.

Introduction

Serum Epitope Repertoire Analysis (SERA) is a universal serology platform that enables unlimited multiplexing in a single test



We are building a high resolution map of the antigenic epitopes associated with an array of human diseases and exposures.

Monitoring humoral immunity in individuals and populations over time may enable earlier diagnoses and reveal cryptic disease associations.

Methods

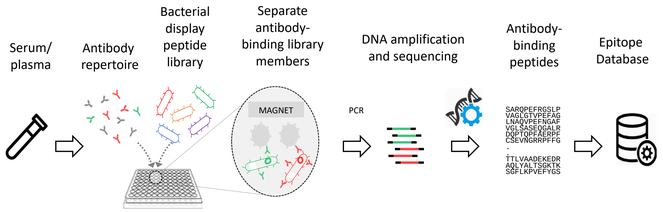


Figure 1. SERA Assay workflow.
Serum is incubated with a bacterial display random 12mer peptide library (diversity 10^{10}) in a 96 well plate format and antibodies bind to expressed peptides that resemble their natural antigen epitopes. Antibodies and their bound bacterial clones are selected with magnetic protein conjugated beads.

Bacterial pools are grown overnight, plasmids encoding the peptides are purified and the peptide encoding region is PCR amplified, barcoded and sequenced on the Illumina NextSeq500, resulting in ~1 million unique reads per sample.

DNA reads are converted to amino acid sequences and uploaded to a database of over 7000 unique epitope repertoires from people with infections, autoimmune disease and cancer as well as healthy people across all age groups and multiple geographies.

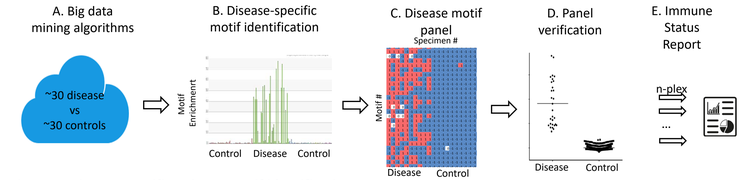


Figure 2. Disease-specific epitope motif identification.
A. The IMUNE algorithm compared disease positive and negative antibody repertoires to discover candidate disease-specific epitope motifs.
B. Candidate motifs were down-selected against a database of controls (n=500-1000) not used for discovery based on sensitivity and specificity.
C. A panel of disease-specific motifs was assembled and a score was derived by converting motif enrichment values to z-scores and summing the scores (composite score).
D. A cutoff (composite score, # of + motifs) was set based on optimal specificity and sensitivity, and the panel was verified on a new set of disease and controls not used for discovery.
E. A composite score for each panel was calculated yielding a comprehensive report for all panels for each patient.

Results

Disease-specific epitope panels can be rapidly discovered and implemented

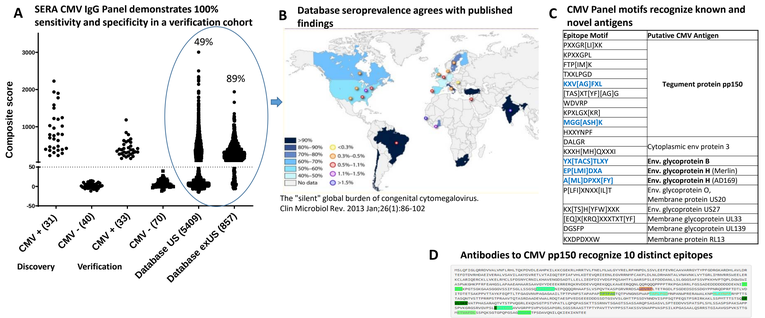


Figure 3. A) A CMV-specific motif panel was discovered and verified on CMV predicate positive and negative serum samples. **B)** Seroprevalence of repertoires from exUS (including S. and C. America, Africa, Europe and Asia) (89%), was significantly higher than seroprevalence in the US (49%), consistent with published estimates[1]. **C)** Twenty of thirty-two motifs mapped to CMV antigens using the SCANPROSITE search engine. Blue denotes motifs with exact matches in the IEDB database. Bold indicates antigen matches in IEDB. Twelve motifs (not shown) had no direct antigen hits. **D)** Amino acid sequence of Tegument protein pp150. IMUNE identified 10 distinct epitopes (green and brown).

SERA differentiates acute disease vs previous exposure: together, Acute IgG & IgM Toxoplasmosis panels are 100% sensitive and highly specific

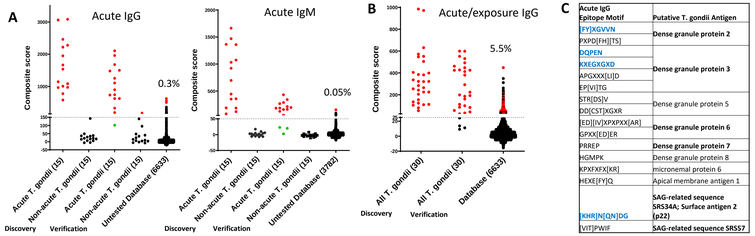


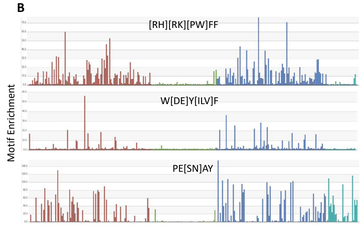
Figure 4. A) Acute Toxoplasmosis IgG & IgM panels were discovered using predicate tested *T. gondii* sera provided by the CDC. The panels were verified in a blinded validation study of 120 sera from several parasitic diseases. Red indicates positive samples (composite score, # of + motifs). Green = negative on the indicated panel but positive on the acute panel for the other isotype. **B**) A chronic IgG panel detects all *T. gondii* sera. Percent seroprevalence in the Serimmune database of untested repertoires is shown as a measure of panel specificity. **C)** A subset of epitopes motifs that mapped to *T. gondii* antigens. Blue denotes motifs with exact epitope matches in the IEDB database. Bold denotes antigen matches in IEDB.

Epitope level serology provides high resolution view of EBV infection

A

EBV chronic/latent Epitope Motif Panel	Putative EBV Antigen
LL[WF]LxxK	Cytoplasmic envelopment protein 3
[RH][RK][PW]FF	EBNA1
GAG[SNA]XXXX[IAV]	EBNA1
PxHRxGx[RK]	EBNA1
DxPPGA	EBNA1, Portal protein
YFEYH	EBNA1
[GA]xADxFE	EBNA1
PSC[IV]GC	EBNA1
PXLXX[LM]QR	EBNA2
RxPx[IV]x[YW]N	EBNA2
W[DE]Y[ILV]F	EBNA2
FPXXWYP	EBNA2
Px[VI]xExxMF	EBNA3
STGPR	EBNA6 (4 repeats), EBNA1
ExGPFE	EBNA4
PYXXYQ	EBNA6
[WAF]XQXTXPR	EBNA6
[VI]PxxAPxK	Envelope glycoprotein B
Kx[RH]xRWx[FW]	envelope glycoprotein GP350
PVXF[ND]K	Glycoprotein 42
K[LI]xHWT	Glycoprotein 42
PNDVxR	small capsomere interacting protein
[AV]TxGAT	small capsomere interacting protein
P[EH]XTXPR	small capsomere interacting protein
PE[SN]AY	Trans-activator protein BZLF1
DPYQXX[WF]	Trans-activator protein BZLF1

B



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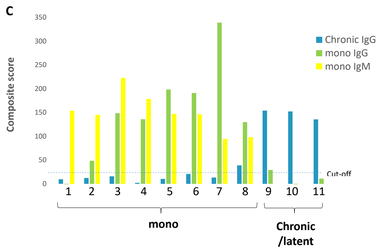
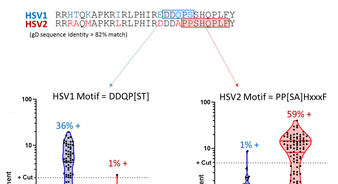


Figure 5. A) 26 epitope motifs recognize 10 distinct EBV antigens. The enrichment of motifs (highlighted in yellow), is shown in motif enrichment plots **(B)**. Each bar on the x axis represents a different patient repertoire from the Serimmune database. Some motifs are specific to EBV chronic/latent (top 2 panels), whereas others are also present in EBV mono (bottom panel). Motifs demonstrate varying sensitivity in EBV positive samples. **D)** EBV chronic/latent, and EBV mono IgG and IgM panels together define different states of EBV infection. Mono samples are positive on mono IgM or G+M, but negative on the chronic/latent EBV panel. Chronic/latent EBV is negative on the mono IgG panel (IgM not done).

Epitope level serology can distinguish antibodies to closely related pathogens

Distinct but overlapping epitope motifs distinguish HSV-1 and HSV-2 in the highly homologous glycoprotein D

A



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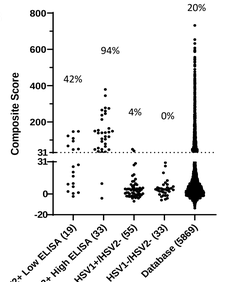

Figure 6. A) The IMUNE algorithm identified specific epitopes in Glycoprotein D of HSV-1 & 2. Glyco D is not suitable to discriminate HSV1 & 2 by ELISA due to high homology. **B)** The HSV-2 motif panel discriminates between HSV-1+/2- and HSV-2+ samples tested by predicate ELISA. HSV-2 positivity is reduced in people with ELISA scores < 3.5 (42% vs 94%). The CDC suggests confirmatory Western Blot tests for ELISA low HSV-2 due to high rates of false positivity[3]. In 5869 untested controls from the Serimmune database, seroprevalence is 20%, higher than published estimates for the US (12%)[4]. The database includes cohorts from around the world as well as STD cohorts, which may partially account for the increased positivity.

Conclusions

SERA is a novel platform that profiles entire antibody repertoires enabling hypothesis free, unlimited serology testing in a single assay.

Panels can be rapidly discovered and evaluated for specificity, leveraging an ever-growing database of human antibody repertoires from diseased and healthy people.

Epitope level serology panels can differentiate acute and chronic/latent infection and discriminate between closely related pathogens.

Immune profiling of individuals over time may allow for earlier diagnoses, and in populations may reveal associations between infections and other diseases including autoimmunity and cancer.

SERA: comprehensive immune monitoring from a single assay

- Distinguishing acute from previous infection can be critical e.g. during pregnancy, under immune compromised conditions and in aging
- SERA provides a method to visualize exposures to human pathogens as they occur throughout an individual's lifetime
- The repertoire is a permanent record that can be queried retrospectively as new tests are developed and compared with subsequent time points

Patient: BLLDH251

Panel Category	Panel Name	Panel Motifs	< Normal Range	Value	Result
Autoimmune	Deamidated gliadin peptide (Celiac disease), IgG	4	19.0 / 1	4.2 / 0	NEG——
Bacterial	Bartonella henselae, IgG	24	20.0 / 2	<7.7 / 0	NEG——
Bacterial	Coxiella burnetii, IgG	25	26.0 / 1	4.5 / 0	NEG——
Bacterial	Helicobacter pylori, IgG	19	15.0 / 1	5.1 / 1	NEG——
Fungal	Coccidioides, IgG	9	50.0 / 1	<2.4 / 0	NEG——
Other	Common Motif, IgG	132	400.0 / 1	1072.1 / 51	——POS
Parasitic	Leishmania, IgG	20	25.0 / 1	5.3 / 1	NEG——
Parasitic	Plasmodium falciparum (Malaria), IgG	12	18.0 / 1	2.3 / 0	NEG——
Parasitic	Taenia solium (Cysticercosis), IgG	27	30.0 / 1	<4.8 / 0	NEG——
Parasitic	Toxocara, IgG	25	25.0 / 1	2.8 / 1	NEG——
Parasitic	Toxocara, IgM	9	10.0 / 2	<2.8 / 0	NEG——
Parasitic	Toxoplasma gondii (acute), IgG	39	150.0 / 6	2.3 / 1	NEG——
Parasitic	Toxoplasma gondii (chronic/latent), IgG	28	25.0 / 2	1.6 / 0	NEG——
Parasitic	Toxoplasma gondii, IgM	10	50.0 / 4	3.1 / 0	NEG——
Parasitic	Trypanosoma cruzi (Chagas disease), IgG	39	50.0 / 2	<0.7 / 0	NEG——
Tick-borne	Anaplasma phagocytophilum, IgG	19	30.0 / 2	<0.4 / 0	NEG——
Tick-borne	Anaplasma phagocytophilum, IgM	11	20.0 / 2	2.1 / 0	NEG——
Tick-borne	Babesia microti, IgG	20	26.0 / 1	0.8 / 0	NEG——
Tick-borne	Babesia microti, IgM	18	21.0 / 2	<3.8 / 0	NEG——
Tick-borne	Borrelia burgdorferi (Lyme disease), IgG	23	28.0 / 1	6.0 / 2	NEG——
Tick-borne	Borrelia burgdorferi (Lyme disease), IgM	43	35.0 / 1	34.0 / 1	NEG——
Tick-borne	Ehrlichia chaffeensis, IgG	11	21.0 / 1	<0.5 / 0	NEG——
Tick-borne	Ehrlichia chaffeensis, IgM	9	10.0 / 1	<2.6 / 0	NEG——
Viral	Chikungunya Virus, IgG	28	20.0 / 1	2.1 / 0	NEG——
Viral	Cytomegalovirus, IgG	31	25.0 / 2	638.4 / 17	——POS
Viral	Epstein-Barr virus (Mononucleosis), IgG	21	25.0 / 2	1.3 / 0	NEG——
Viral	Epstein-Barr virus (Mononucleosis), IgM	9	20.0 / 2	0.6 / 0	NEG——
Viral	Epstein-Barr virus (chronic/latent), IgG	25	25.0 / 2	455.4 / 14	——POS
Viral	Hepatitis C virus, IgG	13	30.0 / 1	<0.3 / 0	NEG——
Viral	Herpes simplex virus 1, IgG	30	50.0 / 1	224.9 / 10	——POS
Viral	Herpes simplex virus 2, IgG	30	40.0 / 1	118.7 / 7	——POS
Viral	Human Immunodeficiency virus 2, IgG	6	20.0 / 1	0.9 / 0	NEG——
Viral	Human Immunodeficiency virus, IgG	8	25.0 / 1	1.9 / 0	NEG——
Viral	Human T-Lymphotrophic virus 1, IgG	12	30.0 / 1	<3.5 / 0	NEG——
Viral	Human T-Lymphotrophic virus II, IgG	9	28.0 / 1	5.6 / 0	NEG——
Viral	Parvovirus B19, IgG	20	30.0 / 1	10.8 / 1	NEG——
Viral	West Nile virus, IgG	30	30.0 / 1	<0.3 / 0	NEG——
Viral	Zika virus, IgG	14	30.0 / 1	5.5 / 1	NEG——
Viral	Zika virus, IgM	21	22.0 / 2	<6.6 / 0	NEG——

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