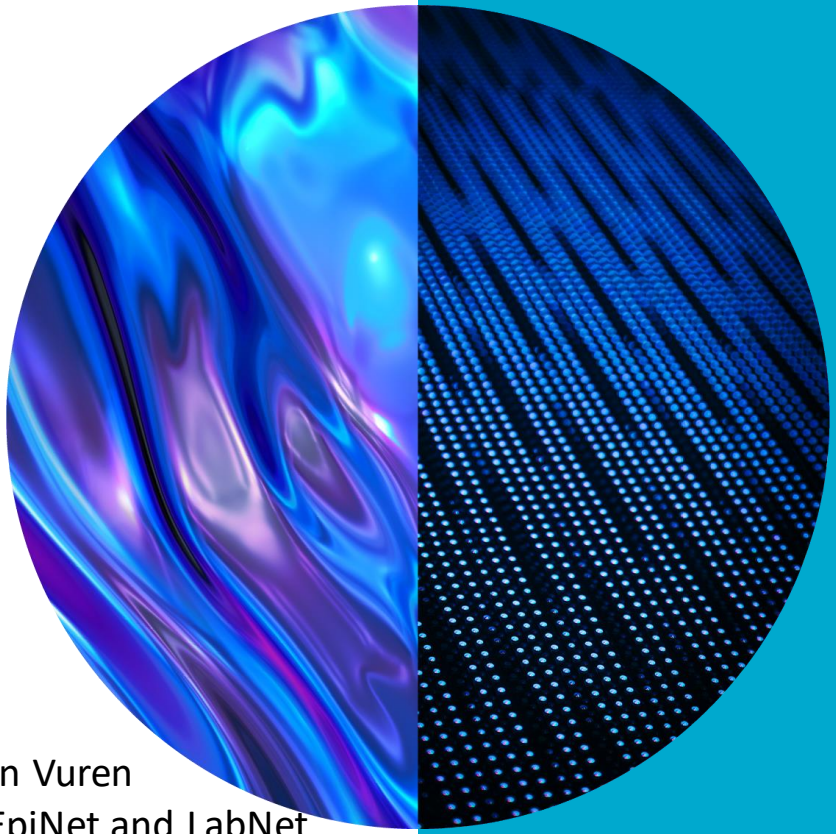
Novel laboratory diagnostic tools for Emergency Animal Diseases including FMD



Petrus Jansen van Vuren

SEACFMD Joint EpiNet and LabNet Virtual Meeting 8 December 2022

Australian Centre for Disease Preparedness

Traditional serology for EADs

- Detection of antibodies to pathogens forms an integral part of the diagnostic process and is essential for surveillance studies
- Inherently difficult to perform and interpret
  - Non-specific cross-reactivity
  - Multi-serotype viruses – cross-reaction
  - Timing of collection
  - Time consuming
  - Gold standard VNTs often require high biocontainment
  - Reagent production requires high biocontainment
  - Lack of standardisation
  - Lack of validation data

Traditional serology for EADs

- Most commonly used:
  - ELISA (different formats e.g. indirect, direct, sandwich, capture, competition) – inactivated whole Ags vs. recombinant Ags
  - Haemagglutination inhibition (HAI)
  - PRNT and VNT
  - Immunofluorescence antibody test (IFAT)
  - Lateral flow devices (field)
  - Luminex (multiplex up to 100 analytes) – emerging technology
- Multiple assays required per single sample in diagnostic setting
- Targeted sero-surveillance (100s to 1000s samples) focuses on a single target (virus and/or serotype) to remain feasible

Filling the gaps requires a unique approach

- Distinguishing cross-reactive antibodies to similar viruses
- Single test vs. multiple tests
- Higher resolution (epitope vs. whole virus/protein antigen)
- Serotyping without VNT in containment
- Syndrome-based diagnostic tool vs. disease/virus specific

Developments in human health research

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Nat Biotechnol. ; 29(6): 535–541. doi:10.1038/nbt.1856.

Application of a synthetic human proteome to autoantigen discovery through PhIP-Seq

H. Benjamin Larman[1,2,3], Zhenming Zhao[3,4], Uri Laserson[1,5,6], Mamie Z. Li[3], Alberto Ciccia[3], M. Angelica Martinez Gakidis[3], George M. Church[6], Santosh Kesari[7], Emily M. LeProust[8], Nicole L. Solimini[3,*], and Stephen J. Elledge[3,*]

Qualitative Profiling of the Humoral Immune Response Elicited by rVSV-ΔG-EBOV-GP Using a Systems Serology Assay, Domain Programmable Arrays

Mariano Sanchez-Lockhart,[1,2] Daniel S. Reyes,[1,2] Jeanette C. Gonzalez,[1] Karla Y. Garcia,[1,2] Erika C. Villa,[3] Bradley P. Pfeffer,[1] John C. Trefry,[4] Jeffrey R. Kugelman,[1] Margaret L. Pitt,[4] and Gustavo F. Palacios[1,5,*]

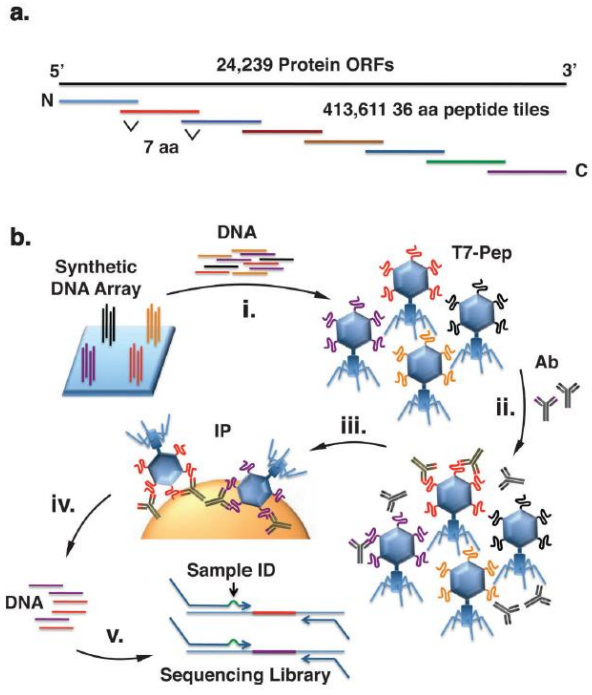
Cell Reports *24*, 1050–1059, July 24, 2018

Comprehensive serological profiling of human populations using a synthetic human virome

SCIENCE
5 JUNE 2015 • VOL 348 ISSUE 6239

George J. Xu, Tomasz Kula, Qikai Xu, Mamie Z. Li, Suzanne D. Vernon, Thumbi Ndung'u, Kiat Ruxrungtham, Jorge Sanchez, Christian Brander, Raymond T. Chung, Kevin C. O'Connor, Bruce Walker, H. Benjamin Larman, Stephen J. Elledge*

Autoimmune disease research

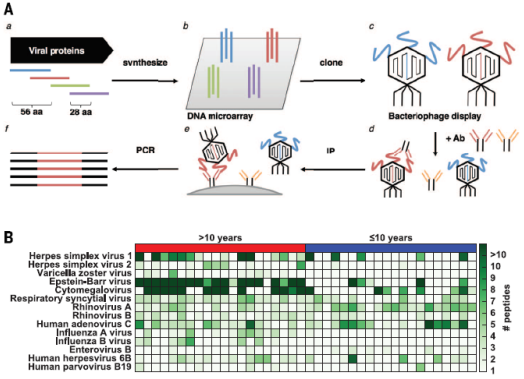


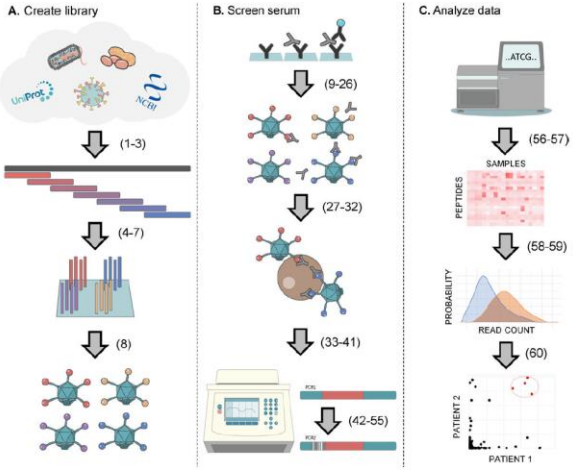
Patient Info	-Log10 P value	Protein	# Peptides	Validation
A: 63 y.o. female with non-small cell lung cancer. Presents with classic cerebellar syndrome. **CSF positive for anti-NOVA antibodies.**	**15.38**	**NEURO-ONCOLOGICAL VENTRAL ANTIGEN 1 (NOVA1)**	**1**	**WB+**
	14.76	**HYPOTHETICAL PROTEIN LOC26080**	**7**	**DB+**
	14.54	**TGFB-INDUCED FACTOR HOMEOBOX 2-LIKE, X-LINKED (TGIF2LX)**	**1**	**WB+**
	8.00	NEBULIN (NEB)	1	NT
	6.49	**DEBRANCHING ENZYME HOMOLOG 1 (DBR1)**	**1**	**WB-,DB+**
	6.20	**PROTOCADHERIN 1 (PCDH1)**	**1**	**WB-,DB+**
	4.29	INSULIN RECEPTOR (INSR)	1	NT
B: 59 y.o. female with non-small cell lung cancer. Presents with dysarthria, ataxia, head titubation and muscle lock. Paraneoplastic antibody panel is negative.	15.18	SOLUTE CARRIER FAMILY 25 MEMBER 43 (SLC25A43)	1	NT
	13.06	**GLUTAMATE DECARBOXYLASE 2 (GAD65)**	**2**	**RIA+,WB-,IP+**
	12.96	**TESTIS EXPRESSED SEQUENCE 2 (TEX2)**	**1**	**DB+**
	12.11	ATAXIN 7-LIKE 3 ISOFORM B (ATXN7L3)	1	NT
	11.93	ETS-RELATED TRANSCRIPTION FACTOR ELF-1 (ELF1)	1	NT
	11.91	**TGFB-INDUCED FACTOR HOMEOBOX 2-LIKE, X-LINKED (TGIF2LX)**	**1**	**WB+**
	11.34	INSULIN RECEPTOR SUBSTRATE 4 (IRS4)	1	NT
	6.98	HEPATOMA-DERIVED GROWTH FACTOR-RELATED PROTEIN 2 (HDGFRP2)	1	NT
	6.60	TUBULIN, BETA (TUBB)	1	WB-
	6.54	**CANCER/TESTIS ANTIGEN 2 (CTAG2)**	**1**	**WB+**
	6.30	**DENN/MADD DOMAIN CONTAINING 1A (DENDD1A)**	**1**	**WB-,DB+**
	6.09	DOUBLESEX AND MAB-3 RELATED TRANSCRIPTION FACTOR (DMRT2)	1	NT
	5.53	TUDOR AND KH DOMAIN CONTAINING ISOFORM A (TDRKH)	1	NT
C: 59 y.o. female with melanoma. Presents with ataxia, dysarthria, horizontal gaze palsy. Paraneoplastic antibody panel is negative. However, CSF stained brain and cerebellar IHC slides.	**15.72**	**TRIPARTITE MOTIF-CONTAINING 67 (TRIM67)**	**2**	**WB+**
	15.65	**TRIPARTITE MOTIF-CONTAINING 9 (TRIM9)**	**3**	**WB+**
	12.13	**FIBROBLAST GROWTH FACTOR 9 (GLIA-ACTIVATING FACTOR) (FGF9)**	**1**	**WB-,DB+**
	10.18	**DUAL-SPECIFICITY TYROSINE-(Y)-PHOSPHORYLATION REGULATED KINASE 3 (DYRK3)**	**1**	**WB-,DB+**
	6.93	CENTROSOMAL PROTEIN 152KDA (CEP152)	1	NT
	6.57	TITIN (TTN)	1	NT
	6.34	NUCLEOPORIN LIKE 2 (NUPL2)	1	NT
	5.43	**HISTONE DEACETYLASE 1 (HDAC1)**	**1**	**WB-,DB+**
	5.36	**MITOCHONDRIAL RIBOSOMAL PROTEIN L39 (MRPL39)**	**1**	**WB-,DB+**
	5.35	**CHROMOSOME 10 OPEN READING FRAME 82 (C10ORF82)**	**1**	**WB-,DB+**
	5.15	NLR FAMILY, PYRIN DOMAIN CONTAINING 5 (NLRP5)	1	NT
	4.83	TASPASE, THREONINE ASPARTASE, 1 (TASP1)	1	NT
	4.70	KIAA0090	1	NT
	4.55	SERINE (OR CYSTEINE) PROTEINASE INHIBITOR, CLADE A (ALPHA-1 ANTIPROTEINASE, ANTITRYPSIN), MEMBER 9 (SERPINA9)	1	NT
	4.21	**PROTEIN TYROSINE PHOSPHATASE, NON-RECEPTOR TYPE 9 (PTPN9)**	**1**	**WB-,DB+**

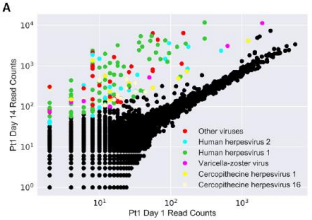
Larman et al., 2011, Nat Biotechnol

Infectious disease research

93,904 peptides from 206 viral species

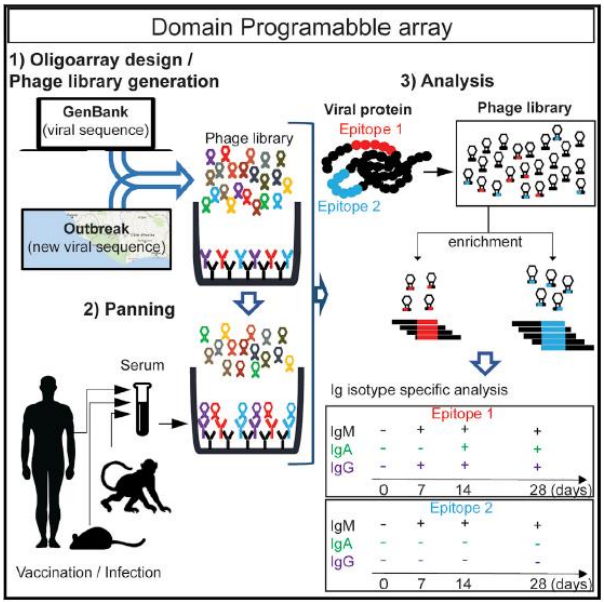


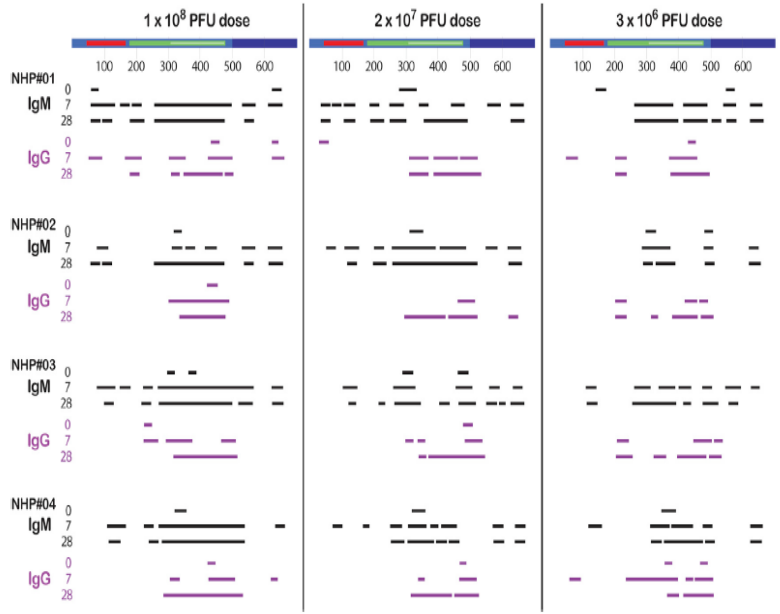




Mohan et al., 2018, Nat Protoc
Xu et al., 2015, Science
Monaco et al., 2018, bioRxiv

Infectious disease research

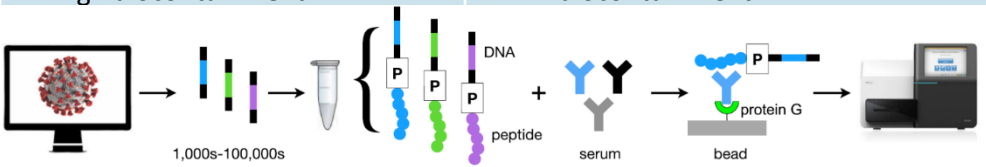




Sanchez-Lockhart et al., 2018, Cell Reports

Infectious disease research

Conventional serology methods	Phage and peptide libraries
• One test per virus	• Single one-pot test (~1 µl serum)
• Non-specific cross-reactivity	• High resolution
• Difficult to perform and interpret	• Simple, standardised workflow
• Time-consuming	• Results in as fast as 2 days
• Viral neutralisation tests require high biocontainment	• Does not require high biocontainment



Design *in silico* | DNA library | Peptide-DNA Library | Binding assay | Sequencing & Analysis

Ladner, J. T. et al. (2021). *Cell Reports Medicine*, *2*(1).

PhiP-Seq vs. traditional approaches

Comparison between the T7-Pep + PhIP-Seq approach and current proteomic methods for autoantigen discovery.

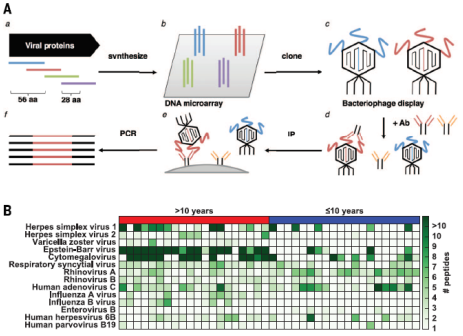
Feature	Classic cDNA Phage Display	Protein Array	T7-Pep + PhIP-Seq
Proteome representation	• Incomplete • Highly skewed distribution	• Small fraction • Uniform distribution	• Nearly complete • Uniform distribution
Fraction of clones expressing an ORF peptide in frame	As low as 6%	Up to 100%	~83%
Size of displayed peptides	Up to full-length proteins	Up to full-length proteins	36 amino acid overlapping tiles
Rounds of selection	Requires multiple selection rounds, which favor more abundant and faster growing clones	No selection	Single selection, which eliminates clone growth bias and population bottleneck
Analysis	Individual clone sequencing: • Initial abundance unknown • Requires population bottleneck	Microarray scanning: • Quantitative • Statistical analysis of antibody binding	Deep sequencing of library: • Quantify population before and after a single round of selection • Statistical analysis of enrichments
Determination of antibody polyclonality	Difficult	Not possible	Often straightforward for antigens of known crystal structure
Epitope mapping	Difficult	Not possible	Often straightforward
Effort	Labor intensive	Minimal	Minimal
Sample throughput	Low	Medium	Adaptable to 96 well format
Multiplexing capability	No	No	Yes
Cost	Low	Moderate to high	Moderate

How can we apply it to improve EAD serology?

- Foot-and-mouth disease
  - Single assay to distinguish between 6 serotypes (serotype C was last recorded in 2004 in the Amazon province, Brazil; is considered extinct)
  - Differentiating Infected from Vaccinated Animals (DIVA) in FMD-free countries when emergency vaccination is considered – SP vs NSP
- Bluetongue disease
  - Single assay to distinguish between serotypes of importance in Australia
- BVDV, BDV, CSF and other diseases caused by pestiviruses of importance
  - Single assay to distinguish between different pestiviruses
- Differential tool for CSF vs. ASF
- Species specific syndromic diagnostic tool

Typical design process and workflow

- Literature review and bioinformatics for design of Oligonucleotide Library Synthesis (OLS)
  - FMD all 7 serotypes, 6 proteins
    - A, O, Asia-1, C, SAT1, 2 and 3
    - VP1 (1D); VP2 (1B); VP3 (1C); VP4 (1A); 2B; 3ABC
- Collapse FMD sequences to 85% identity (serotype differentiation threshold at aa level)
- pepsyn tool used:
  - peptide length 36 aa
  - tile every 7 aa (thus overlap 29 aa)
  - remove duplicate peptides (100% identity, cd-hit)
  - reverse translate peptides to nucleic acid sequences
    - *E. coli* codon optimised



- Immunoprecipitation reaction (serum + phages + protein A/G magnetic beads)
- Perform barcoding and sequencing of enriched phages
- Determine enriched peptides to characterise reactive antibodies in serum sample

Acknowledgements

- Wilna Vosloo
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  - Cattle Compensation Fund – Agriculture Victoria
  - FMD Ready Project