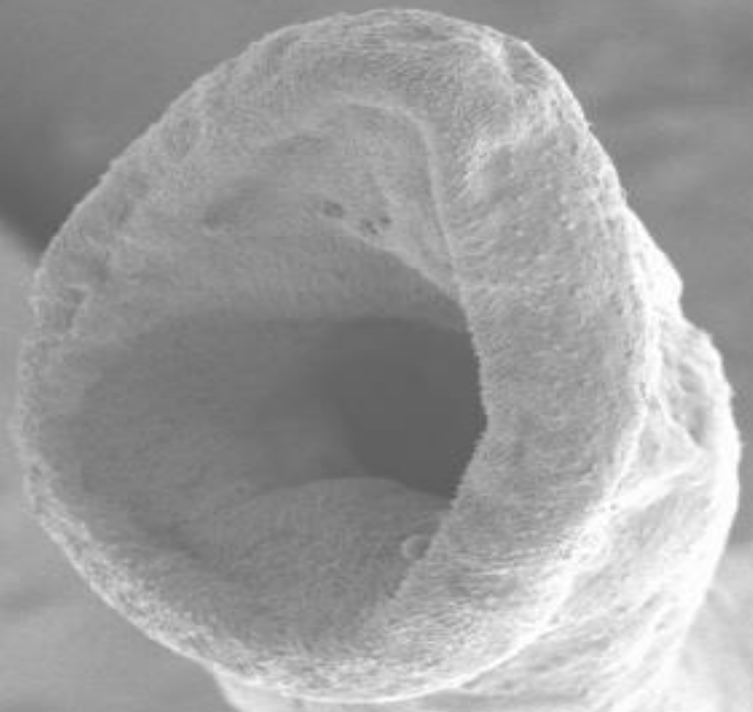
Discovery of *S. haematobium* diagnostic antigens using an immunomics approach



Alex Loukas

Australian Institute of Tropical Health & Medicine

James Cook University, Australia

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Diagnosis of *S. haematobium* infection

- Gold standard is microscopic detection of eggs in urine.
  - low sensitivity – light infections misdiagnosed
  - won't detect early or non-patent infections
- Circulating antigen (eg: CCA and CAA) detection is more sensitive
  - sensitivity/specificity issues in *Sh* infection (Sanneh 2017) and *Sm* infection in Brazil (Bezerra 2018)
- Urine PCR diagnostics
  - sensitivity/specificity is high
  - expensive (DNA needs to be extracted) and not easily field deployable
- ELISA detection of *Sh* SEA.
  - mostly performed with serum
  - Complex mixture (including glycans) so specificity reduced
  - SEA is not a renewable reagent (QC/QA issues)
- Requirement for sensitive and specific antibody PoC test using renewable reagents to complement antigen detection test.

Protein microarrays allow high throughput antigen discovery from helminth proteomes

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PLOS | PATHOGENS

An Immunomics Approach to Schistosome Antigen Discovery: Antibody Signatures of Naturally Resistant and Chronically Infected Individuals from Endemic Areas

Soraya Gaze[1,2], Patrick Driguez[3], Mark S. Pearson[1], Tiago Mendes[4], Denise L. Doolan[3], Angela Trieu[3], Donald P. McManus[3], Geoffrey N. Gobert[3], Maria Victoria Periago[2], Rodrigo Correa Oliveira[2], Fernanda C. Cardoso[3,5], Guilherme Oliveira[2], Rie Nakajima[6], Al Jasinskas[6], Chris Hung[6], Li Liang[6], Jozelyn Pablo[6], Jeffrey M. Bethony[7], Philip L. Felgner[6], Alex Loukas[1]*

Immunology & Cell Biology

Australasian Society for Immunology Inc.

Original Article

Specific humoral response of hosts with variable schistosomiasis susceptibility

Patrick Driguez, Hamish EG McWilliam, Soraya Gaze, David Piedrafita, Mark S Pearson, Rie Nakajima, Mary Duke, Angela Trieu, Denise L Doolan, Fernanda C Cardoso, Algis Jasinskas, Geoffrey N Gobert, Philip L Felgner, Alex Loukas, Els Meeusen, Donald P McManus, ... See fewer authors

frontiers in Immunology

ORIGINAL RESEARCH
published: 05 May 2015
doi: 10.3389/fimmu.2015.00213

Of monkeys and men: immunomic profiling of sera from humans and non-human primates resistant to schistosomiasis reveals novel potential vaccine candidates

*Mark S. Pearson[1], Luke Becker[1], Patrick Driguez[2], Neil D. Young[3], Soraya Gaze[4], Tiago Mendes[5], Xiao-Hong Li[6], Denise L. Doolan[2], Nicholas Midzi[7], Takafira Mduluza[8], Donald P. McManus[2], R. Alan Wilson[9], Jeffrey M. Bethony[10], Norman Nausch[11], Francisca Mutapi[11], Philip L. Felgner[12] and Alex Loukas[1]**

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Edited by:
Rashika El Ridi,

The Journal of Infectious Diseases

MAJOR ARTICLE

Antibody Signatures Reflect Different Disease Pathologies in Patients With Schistosomiasis Due to *Schistosoma japonicum*

Patrick Driguez,[1] Yuesheng Li,[1,4] Soraya Gaze,[2,5] Mark S. Pearson,[2] Rie Nakajima,[6] Angela Trieu,[1] Denise L. Doolan,[1] Philip L. Felgner,[6] Xunya Hou,[4] Fernanda C. Cardoso,[1,3] Algis Jasinskas,[6] Geoffrey N. Gobert,[1] Alex Loukas,[2] and Donald P. McManus[1]

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Succinctus

A next-generation proteome array for *Schistosoma mansoni*

CrossMark

Rafael Ramiro de Assis[a,c], Fernanda Ludolf[b], Rie Nakajima[c], Al Jasinskas[c], Guilherme C. Oliveira[d], Philip L. Felgner[c], Soraya T. Gaze[a], Alex Loukas[e], Philip T. LoVerde[f], Jeffrey M. Bethony[a,g,*], Rodrigo Correa-Oliveira[a], Carlos E. Calzavara-Silva[a,c]

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nature genetics

NATURE GENETICS VOLUME 46 | NUMBER 3 | MARCH 2014

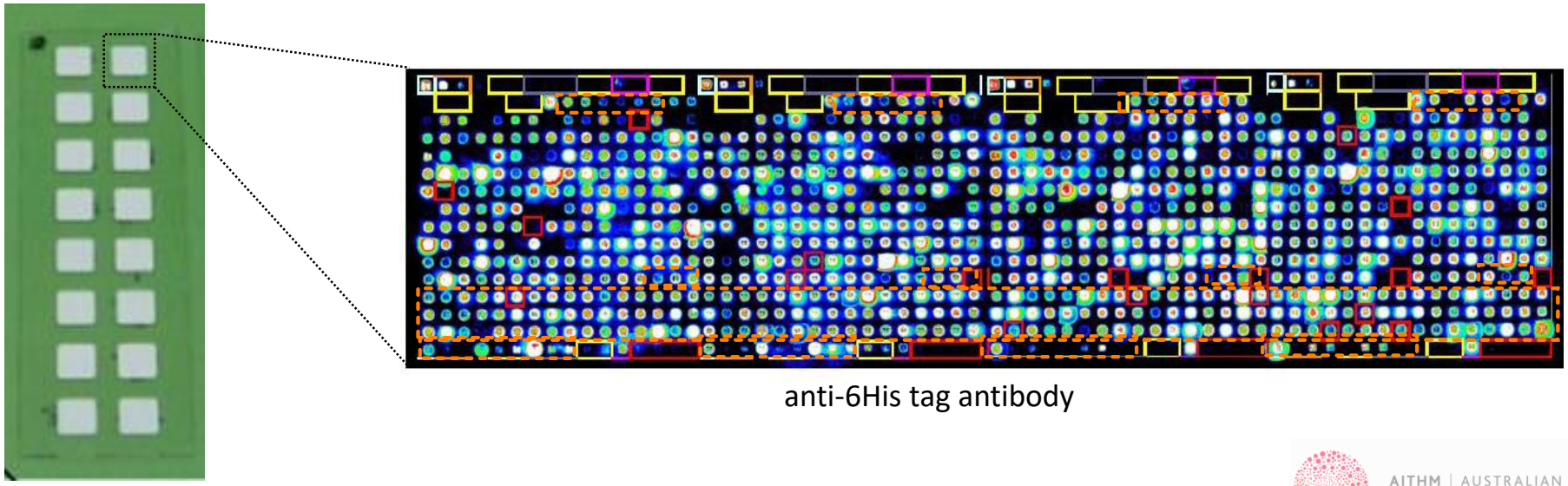
Genome of the human hookworm *Necator americanus*

Yat T Tang[1,16], Xin Gao[1,16], Bruce A Rosa[1,16], Sahar Abubucker[1], Kymberlie Hallsworth-Pepin[1], John Martin[1], Rahul Tyagi[1], Esley Heizer[1], Xu Zhang[1], Veena Bhonagiri-Palsikar[1], Patrick Minx[1], Wesley C Warren[1,2], Qi Wang[1], Bin Zhan[3,4], Peter J Hotez[3,4], Paul W Sternberg[5,6], Annette Dougall[7], Soraya Torres Gaze[7], Jason Mulvenna[8], Javier Sotillo[7], Shoba Ranganathan[9,10], Elida M Rabelo[11], Richard K Wilson[1,2], Philip L Felgner[12], Jeffrey Bethony[13], John M Hawdon[13], Robin B Gasser[14], Alex Loukas[7] & Makedonka Mitreva[1,2,15]

Goals

- Identify antigens to diagnose *S. haematobium* infections by antibody profiling of urine and serum of infected individuals using protein microarrays

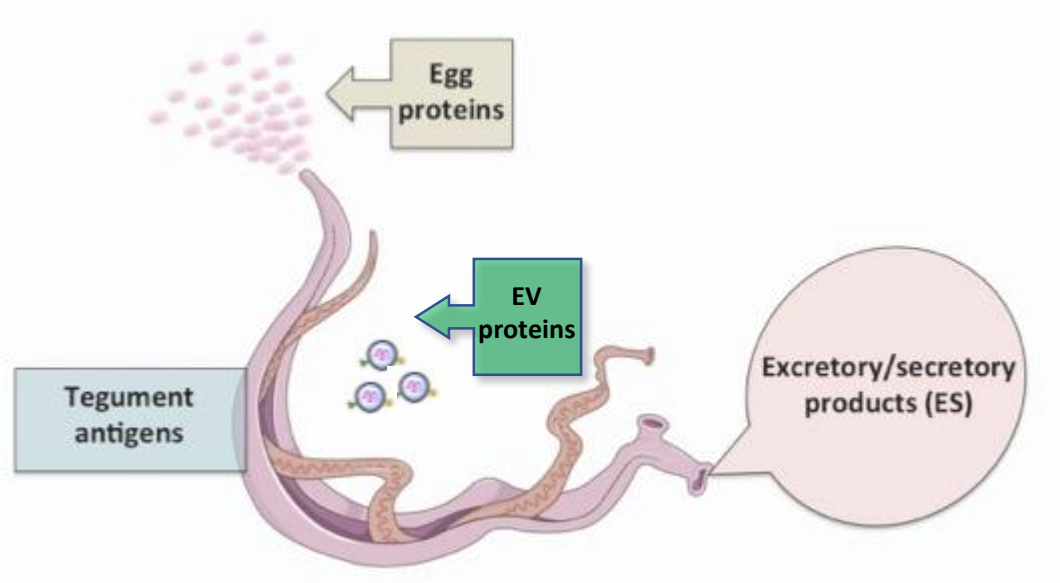
- Use this information to select antigens for downstream development of an antibody-based PoC diagnostic test for *S. haematobium*, ideally using urine as the diagnostic fluid



anti-6His tag antibody

Selection of proteins for inclusion on the array

- Using a mixture of proteome datasets and bioinformatic analyses, 1,053 proteins were selected
- Parasite extracts (including SEA) incorporated into the array as +ve controls

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Succinctus

A next-generation proteome array for *Schistosoma mansoni*

Rafael Ramiro de Assis[a,c], Fernanda Ludolf[b], Rie Nakajima[c], Al Jasinskas[c], Guilherme C. Oliveira[d], Philip L. Felgner[c], Soraya T. Gaze[a], Alex Loukas[e], Philip T. LoVerde[f], Jeffrey M. Bethony[a,g,*], Rodrigo Correa-Oliveira[a], Carlos E. Calzavara-Silva[a,c]

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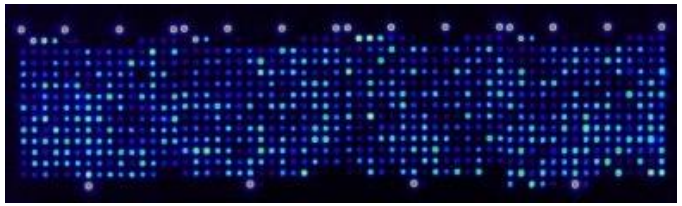
- 650 *S. haematobium* proteins + 403 proteins (*Sh* homologues of *S. mansoni* proteins)
- >90% success in antigen production



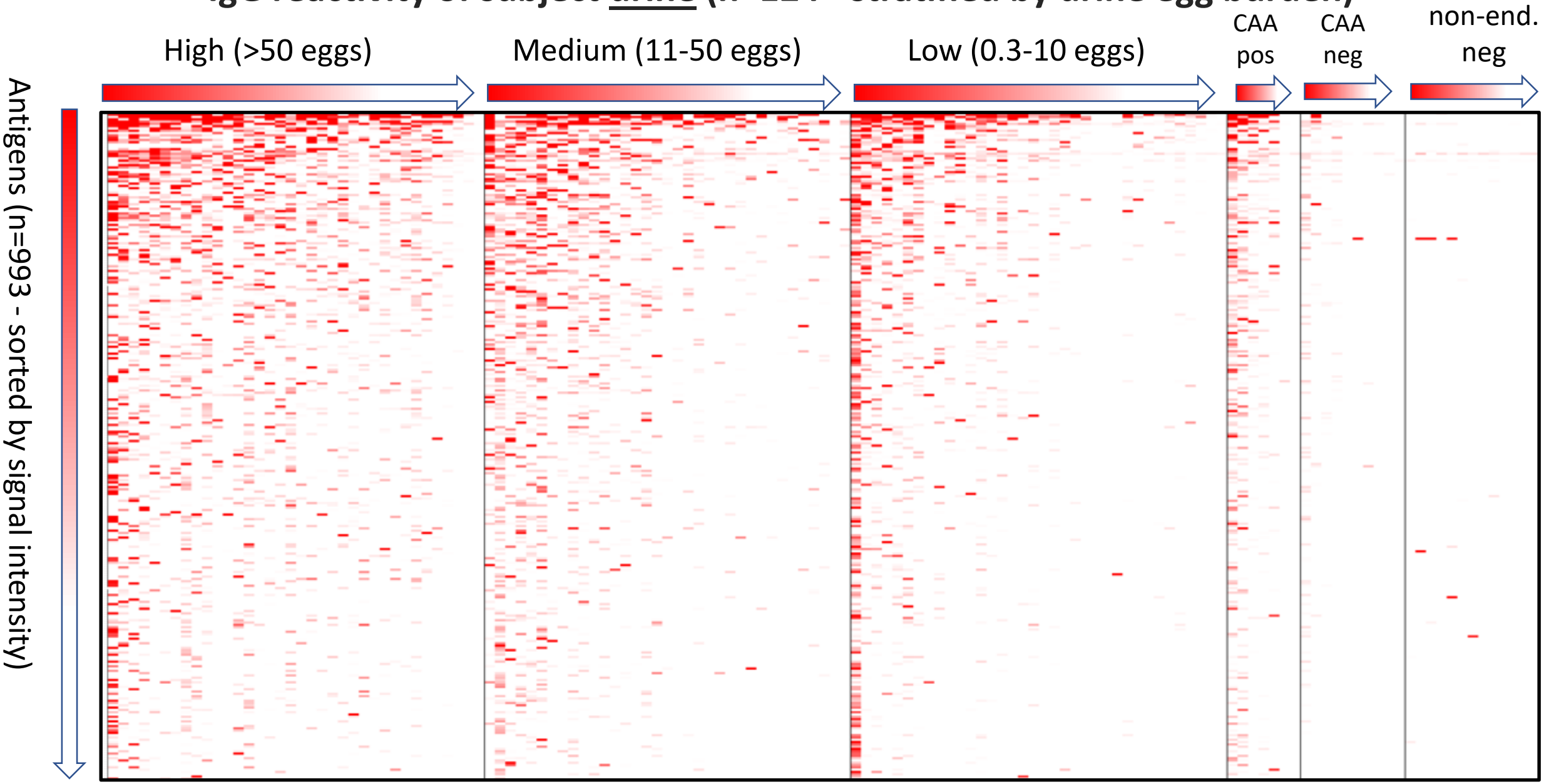
Characteristics of study cohorts

Urine		Sera
124 urine samples from endemic (Sh only) areas of Zimbabwe and Ghana		259 serum samples from endemic (Sh only) areas of Zimbabwe and Ghana
n=36 **high** intensity infections (>50 eggs/10ml urine)		**n=76** high intensity infections (>50 eggs/10ml urine)
n=35 **medium** intensity infections (11-49 eggs/10ml urine)		**n=67** medium intensity infections (11-49 eggs/10ml urine)
n=36 **low** intensity infections (0.3-10 eggs/10ml urine)		**n=98** low intensity infections (0.3-10 eggs/10ml urine)
n=7 **egg negative** infections/**CAA positive** infections		**n=4** egg negative/CAA positive infections
n=10 **egg negative/CAA negative** infections (**endemic** negative controls)		**n=14** egg negative/CAA negative infections (endemic negative controls)
n=13 **non-endemic** negative controls		**n=15** non-endemic negative controls

Aim 1

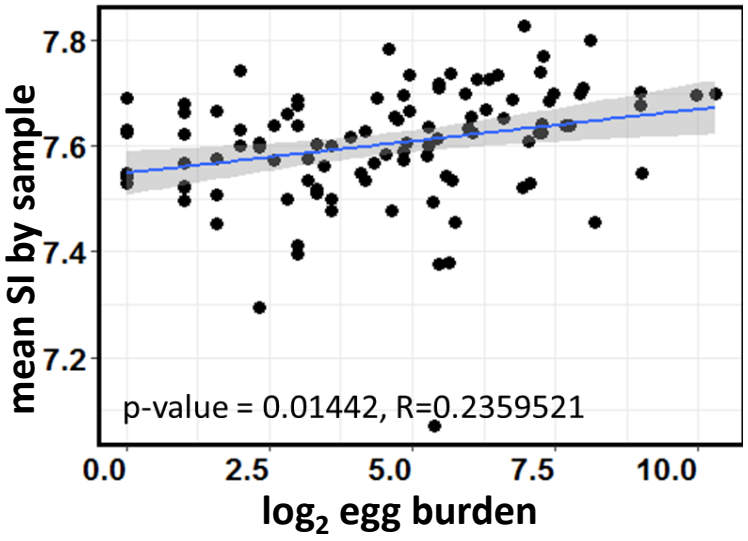
Identification of antibodies from serum/urine that are detectable in all infected cohorts

IgG reactivity of subject urine (n=124 - stratified by urine egg burden)

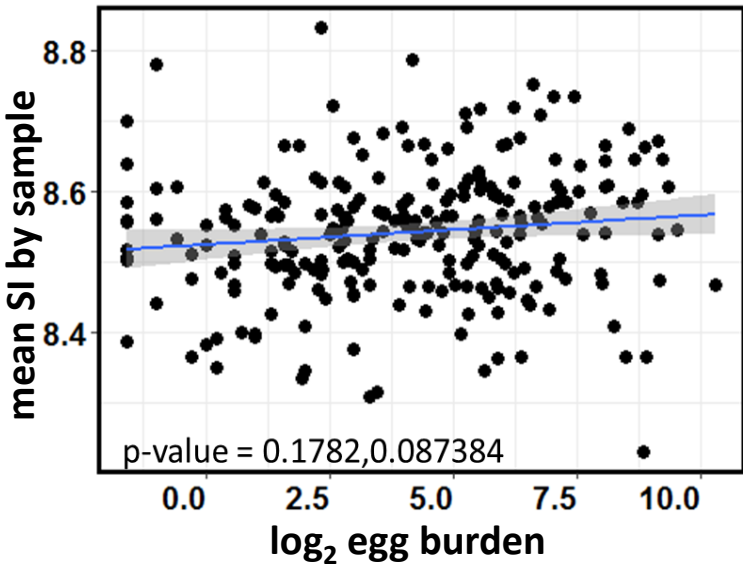


Correlation between egg burden and antibody reactivity in serum and urine

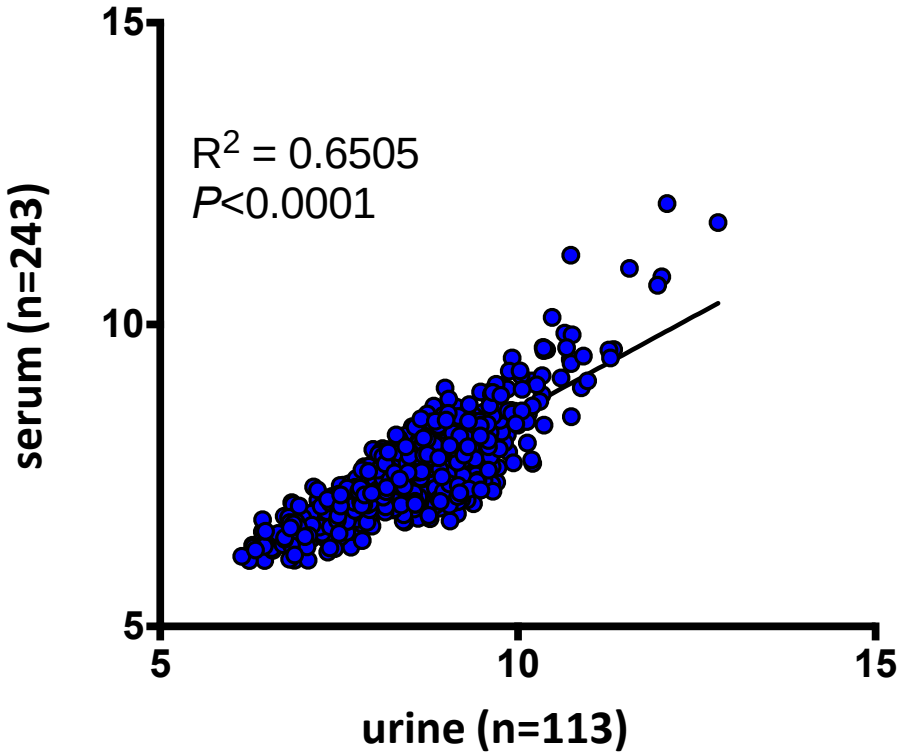
urine



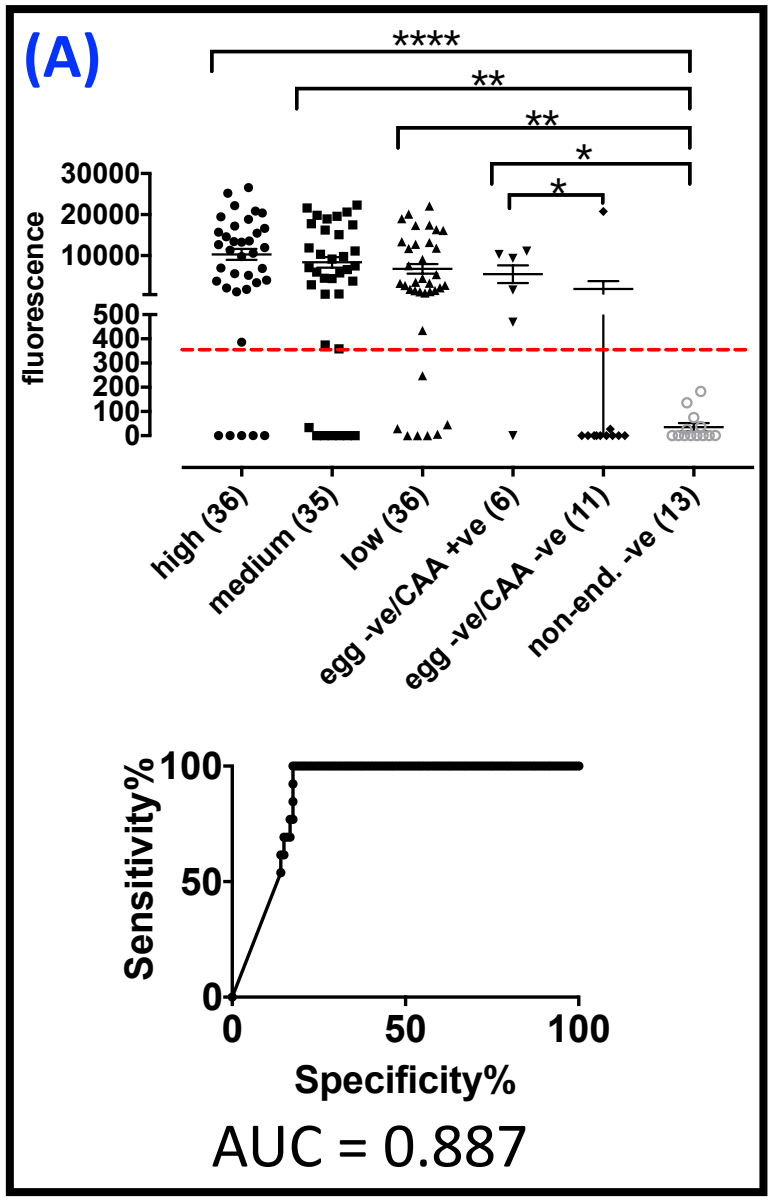
serum

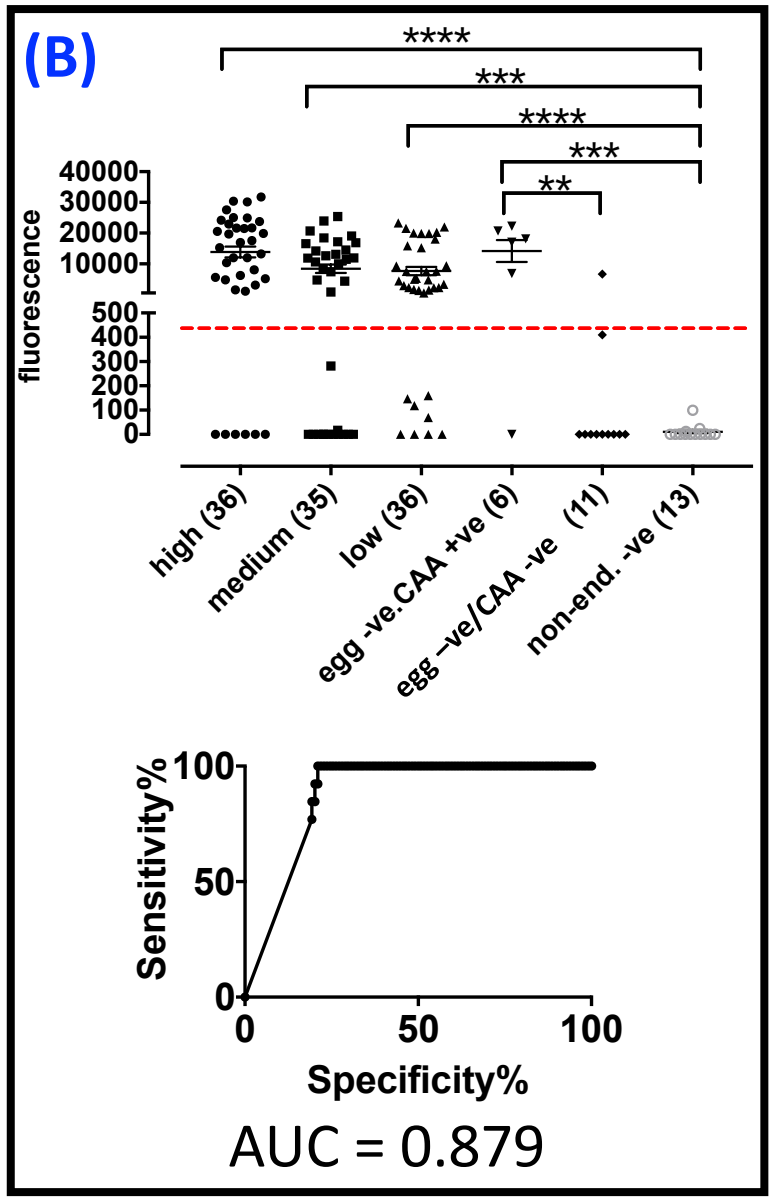


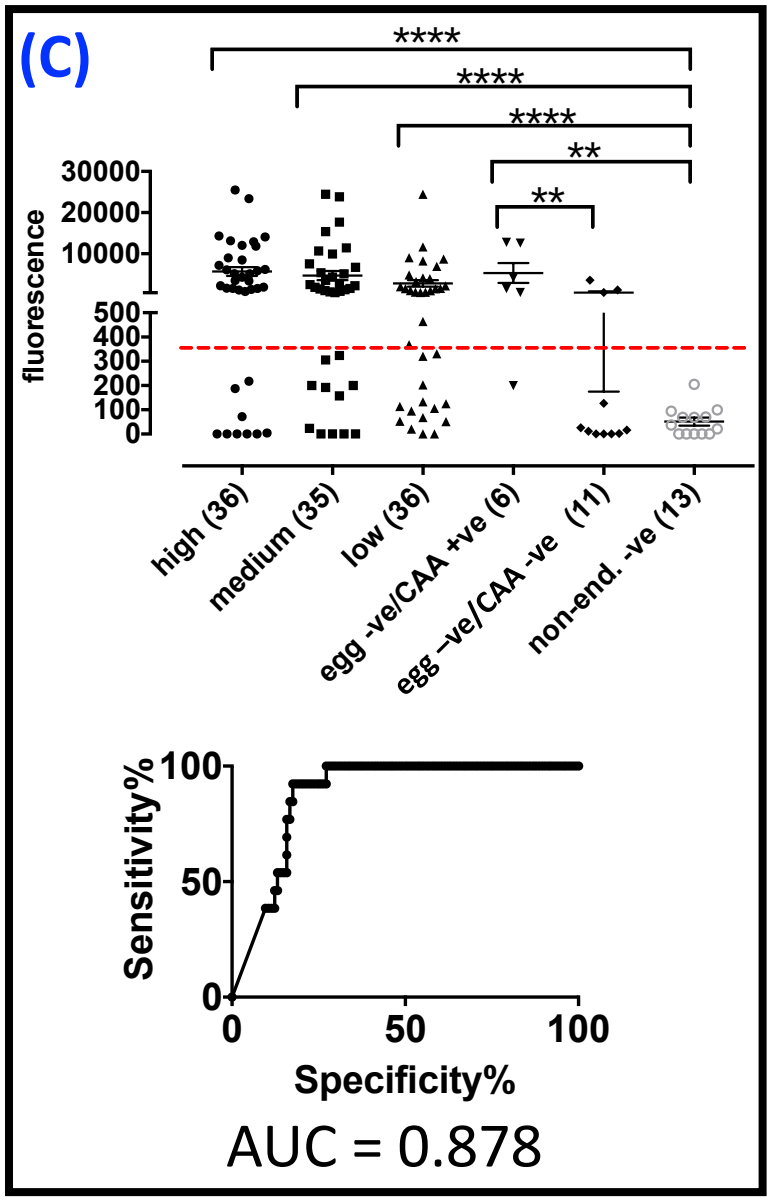
mean SI by antigen
all positive samples



Array reactivity plots and ROC curves of top three antigens (urine)

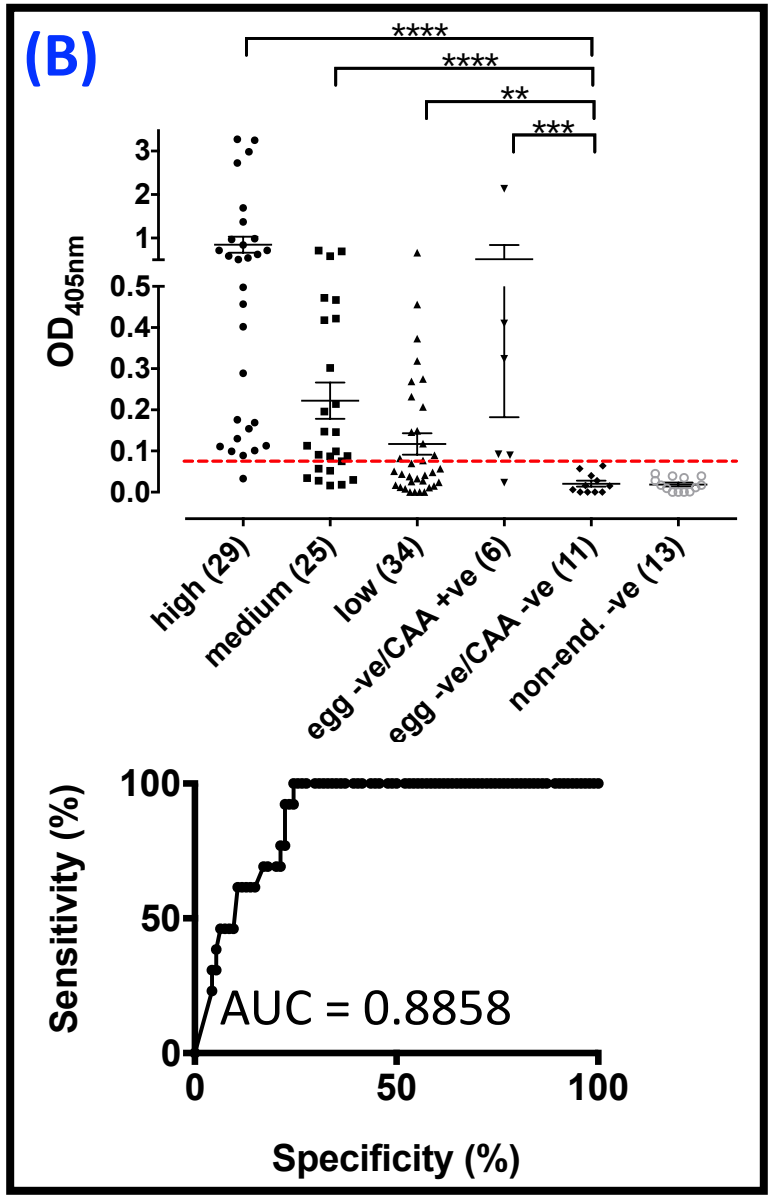


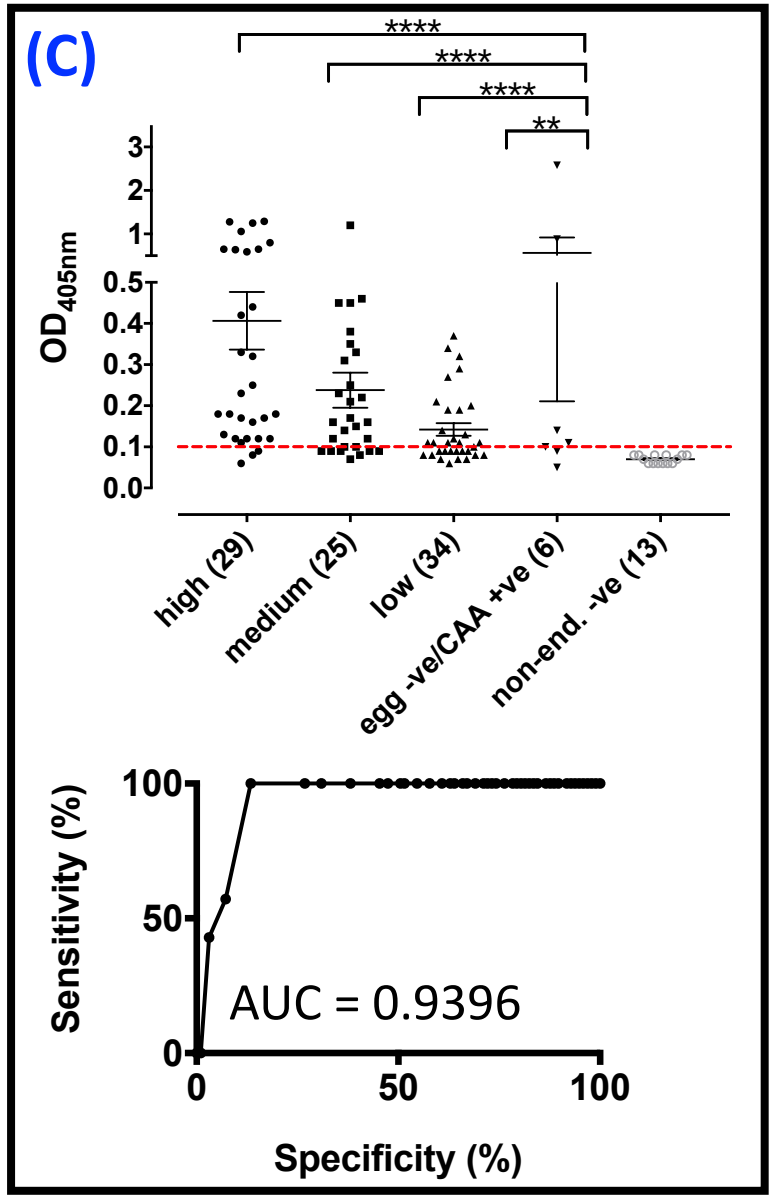




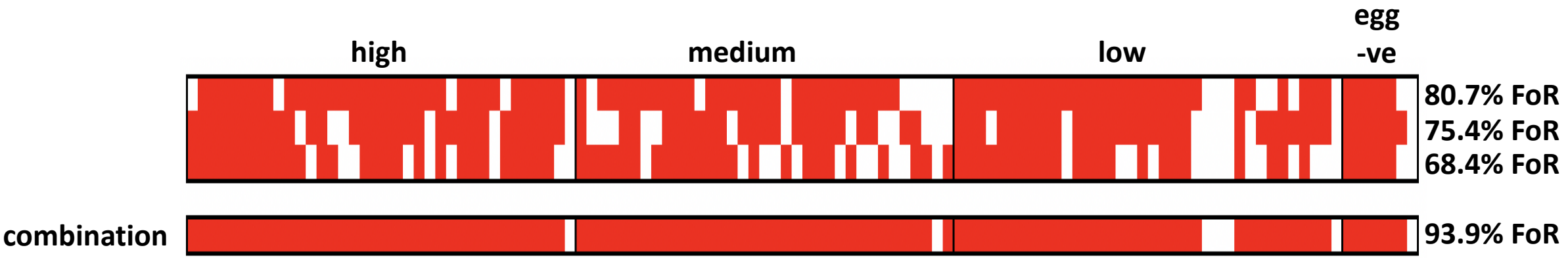
ELISA validation of recombinant antigens B and C (urine)

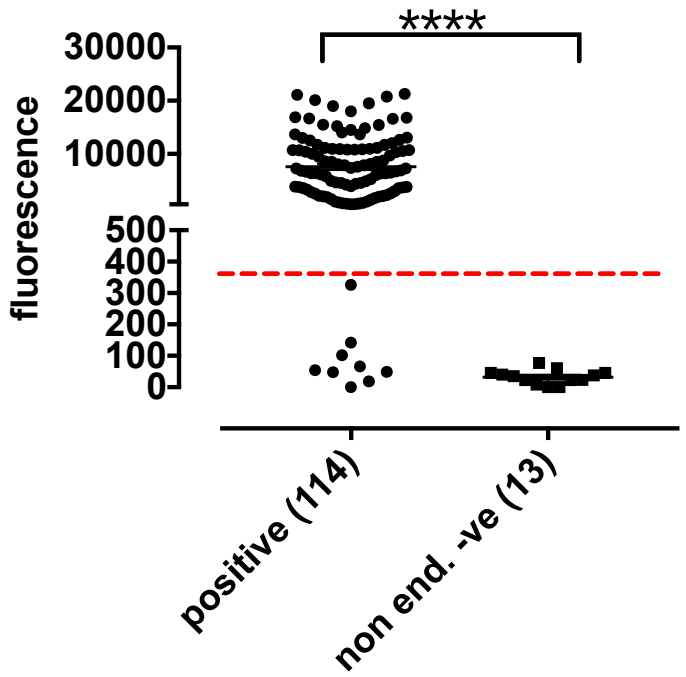
- Cell-free antigens ideal for high-throughput discovery but not scale-up
- Lead antigens expressed in cell-based recombinant systems (yeast and *E. coli*)

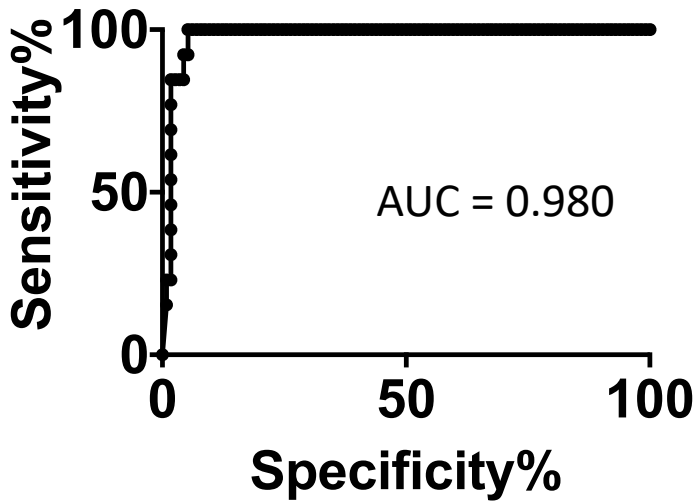




Antigen combinations increase FoR and predictive value of infection (urine)







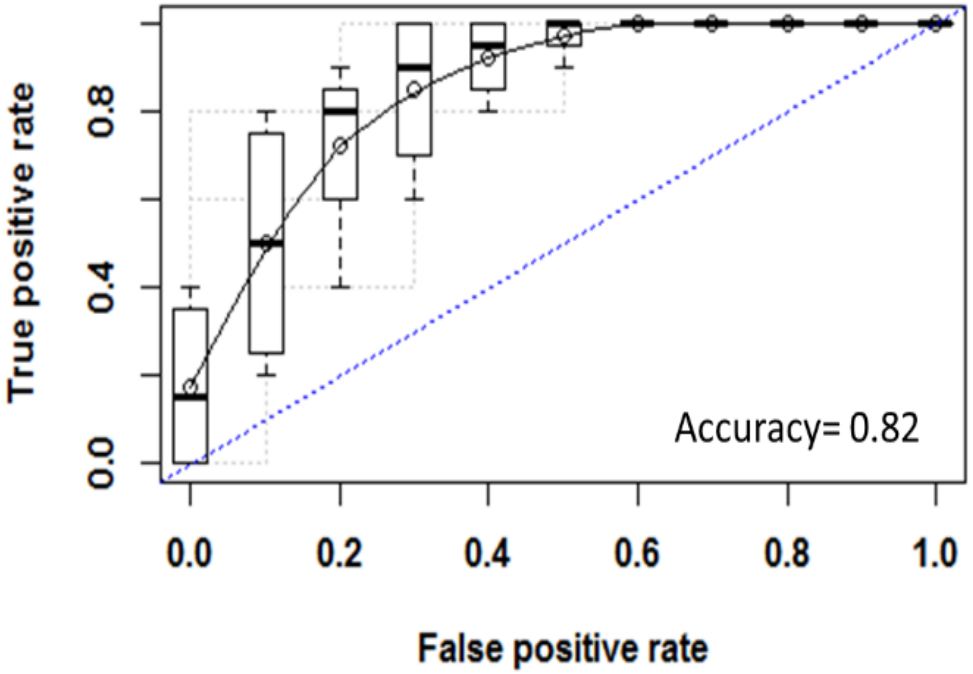
Aim 2

Identify a minimal antibody signature from serum and/or urine which could discriminate between individuals with very low intensity infection (CAA positive, egg negative group) and no infection (CAA negative, egg negative group).

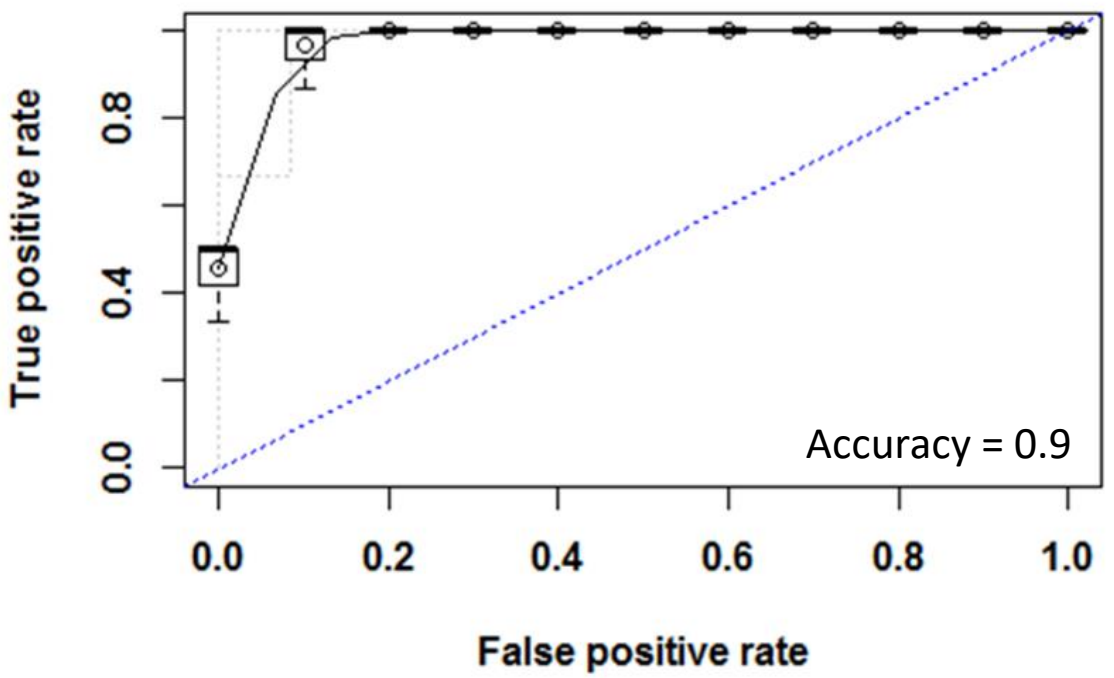
Urine and serum antibody signature verification

- Predictive performance of each signature was examined by averaging ROC characteristics obtained from a 15-fold leave-one-out cross-validation

urine (5 antibody signature)



serum (4 antibody signature)



- SEA accuracy (urine) = 0.85 and SEA accuracy (serum) = 0.77

Summary

- *S. haematobium* protein array (993 antigens) probed with urine (n=137) and sera (n=259) from infected and non-infected individuals.
- In both urine and serum probes, numerous antigens significantly reactive between infected and non-infected populations.
- Diagnostic “signatures” comprised of top-ranked discriminatory antigens have predictive values of infection that exceed SEA, the current ELISA gold standard.
- These antigen signatures can be produced in the lab (more straightforward and rigorous manufacturing process than SEA) and used as the basis for a PoC antibody detection test.

What's next?

- Antigens are being produced in yeast and *E. coli* to validate proteome array results by ELISA
- Kinetics of antibody responses after treatment
- Given the robust association of *S. haematobium* infection with urogenital cancer, can we use arrays to find an immune signature that predicts progression to malignancy?
- Carefully consider a(with GSA consultation) the TPP of an antibody-based diagnostic test for use in elimination setting

- Need access to more samples (*Sh* endemic area) with matching urine/serum samples and circulating antigen test data

People

Mark Pearson

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Beatrice Greco

Maria Elena Bottazzi

Bin Zhan

Zhu Yun,

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Francisca Mutapi

Janice Murray

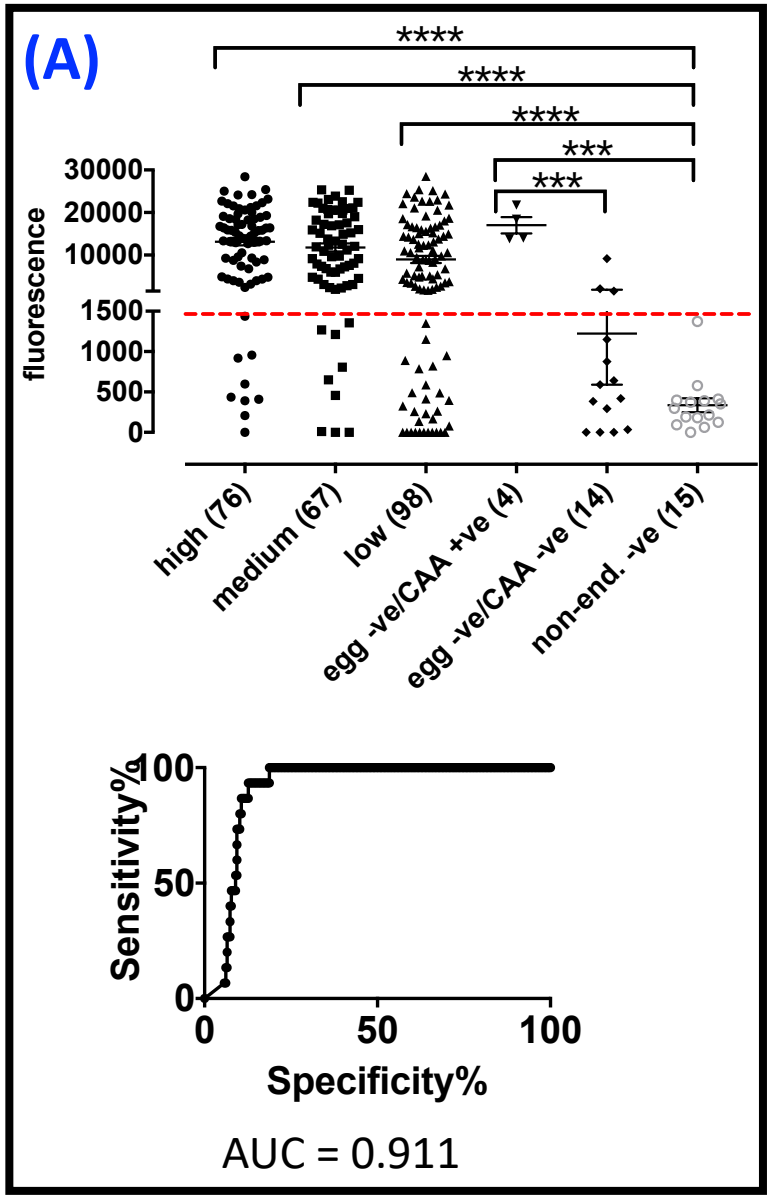
Taka Mduluza

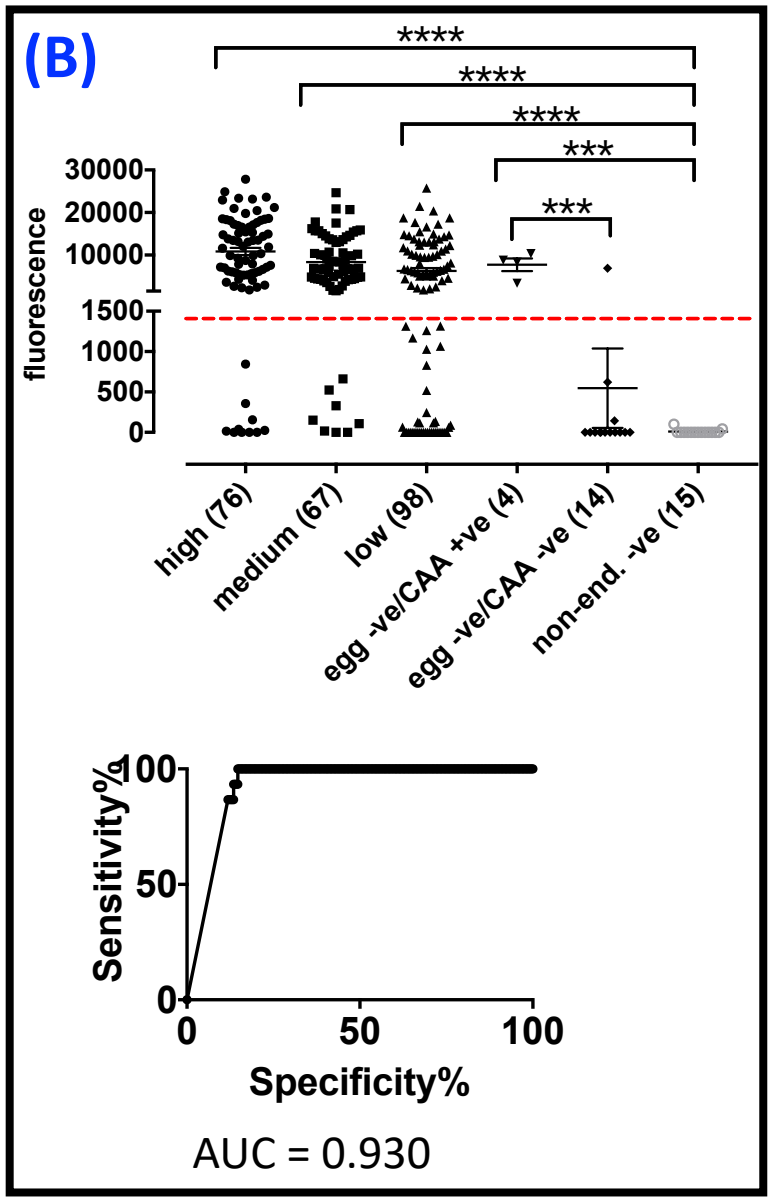
Maria Yazdanbakhsh

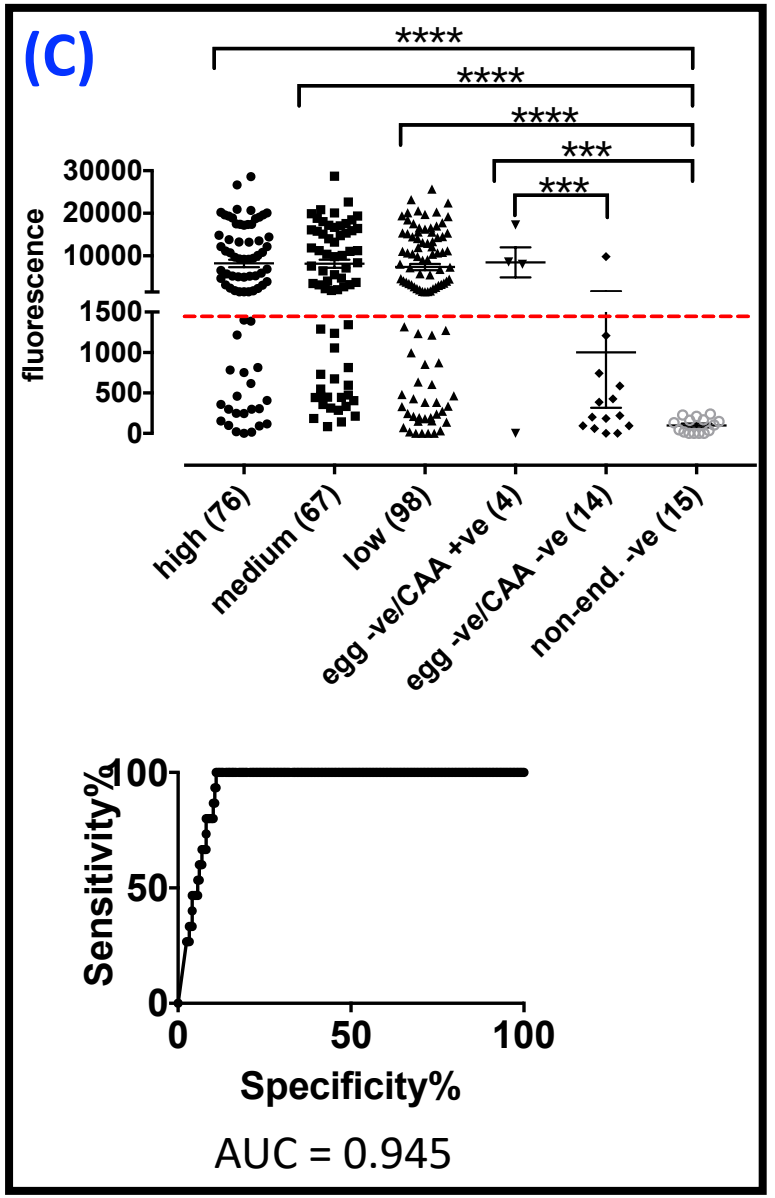
Abena Amoah

Funding

Array reactivity plots and ROC curves of top three antigens (serum)







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Hierarchical clustering (significantly reactive antigens)

Urine