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DISCOVERY OF ANTIBODIES TO *EIMERIA* INFECTIONS IN POULTRY USING NEXT GENERATION PHAGE DISPLAY

Mary Angani[1], Kevin Gough[1], Ben Maddison[2], Richard Emes[1]
University of Nottingham[1],
ADAS, UK[2]

Outline

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- Hypothesis
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- Methods
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Terms

1) Phagemid: Cloning vector that has plasmid and bacteriophage properties used in phage display

2) ScFv (Single chain fragment variable): fusion protein of the variable regions of the heavy and light chains of immunoglobulins. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions

3) HCDR3: Heavy chain complementarity determining region 3 ;part of immunoglobulin and most variable region

Introduction

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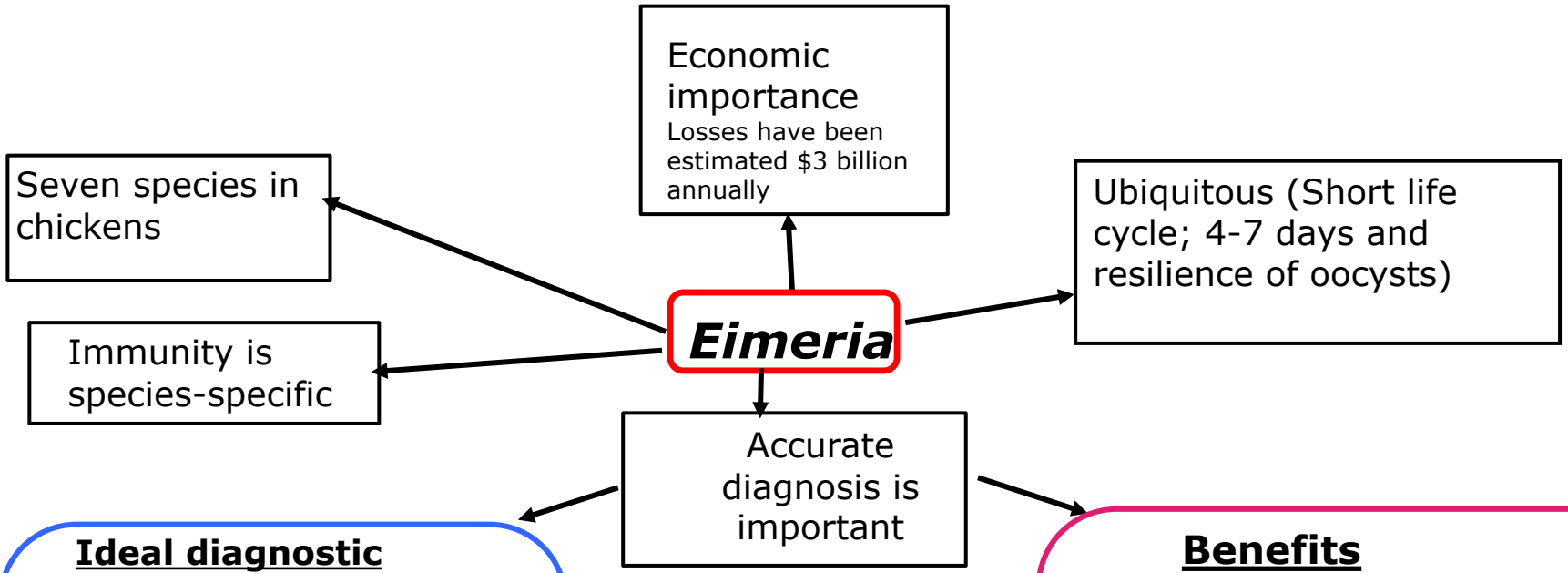

Ideal diagnostic

- Inexpensive
- Sensitivity
- Specificity
- Fast
- Minimum flock handling time
- Minimum sample processing time
- Simple/not requiring expertise

Benefits

- Species prevalent in a flock (evaluate disease risk)
- Reduce vaccine costs
- Investigation of disease outbreaks
- Investigate vaccination efficiency
- Evaluate effectiveness of control programme

Introduction

Phage display

- G. Smith (1985)
- Display peptides and proteins on phage
- Links genotype and phenotype through the phage coat protein
- Hugely successful for the development of monoclonal antibodies
- Starts with antibody library preparation
- Affinity selection technique

Conventional phage display

- Handpicking of clones
- Traditional phage panning: Screen ~100 ligands by ELISA against a single target

Next generation phage display

- Deep sequencing of genes
- NGPD: Screen 10,000's ligands against multiple targets

Hypothesis

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- Next generation phage display will allow comparative screening of antibody binding events to identify antibodies to species-specific epitopes of *Eimeria* oocysts. Such ligands can then be used to develop immunoassays to differentiate infecting *Eimeria* species of poultry.

Aim

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- To isolate antibodies to oocysts to develop in-field species-specific diagnostic assays for *Eimeria* infection

Using Next generation phage display technology [NGPD]

Objectives

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- To carry out antibody-phage display panning against oocysts
  - Isolate antibodies against oocysts of all seven *Eimeria* spp
  - To screen the antibodies isolated via ELISA
- To compare traditional phage panning with Next generation phage display (NGPD)

Methods(NGPD)

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1 Initial Library Griffin 10^{11-13} pfu/mL

2 Incubate With target

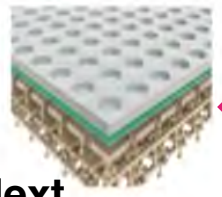
3 Wash off non specific phage

4 Elution of bound phage

5 Sub library $x10^{4-7}$ pfu/mL

Steps 2-6 repeated 2-5 times

6 Infect TG1 with phage to amplify

7  Next generation sequencing

8 *DHTPKWq*
SRSKNYLWRSM
VADPYLAWFRq
Antibody genes

9  Computational Analysis

10  Binding assay

Methods

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- The project began with oocysts
  - Sourcing of oocysts (MSD Animal health)
  - Counting of oocysts (7.0 x 10^5-6.60 x 10^6/ml)

- Griffin Phage library grown 10^{12} PFU/ml
- Target antigen Oocysts 10^4

- Panning experiments with seven species with Griffin library
  - Five rounds of panning, 5 phage and Griffin library(41 samples)

Biopanning strategy

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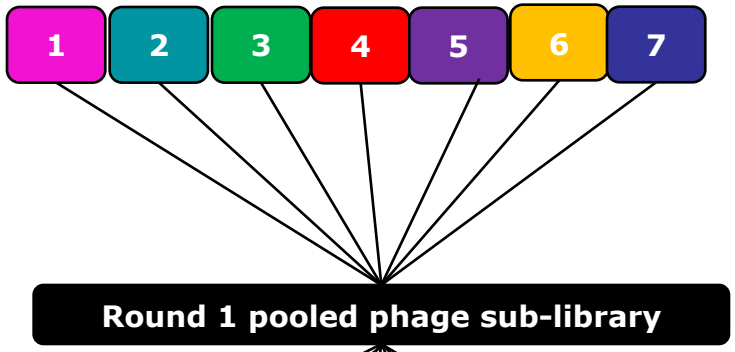
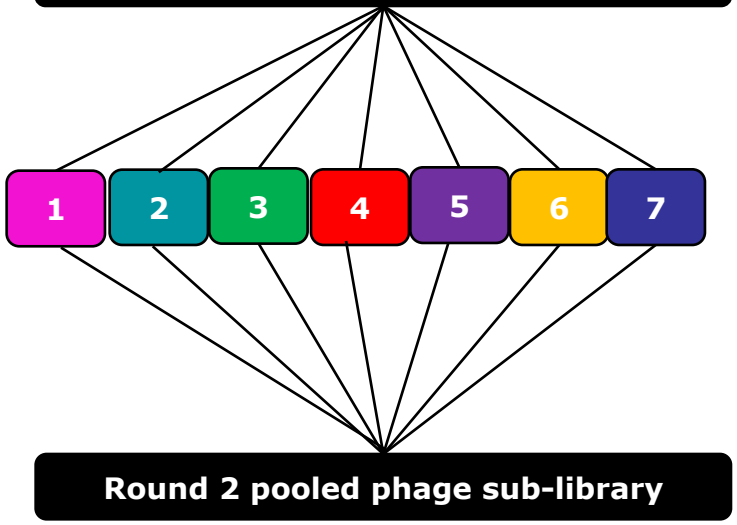
Round 1



Round 2



1-7 represent the seven species of *Eimeria*

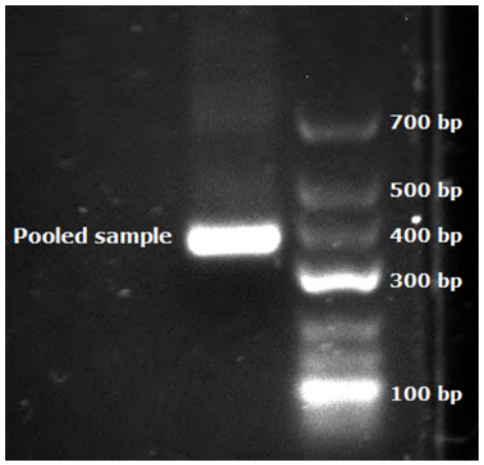
1st round of panning; Antibody library was panned against Individual species of *Eimeria*

Elution by PH shift

A total of 5 rounds of biopanning was carried out

The HCDR3 region was amplified

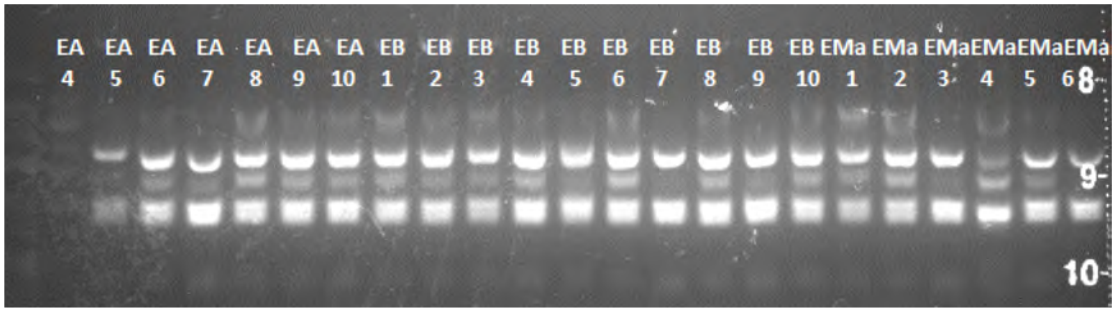
PCR amplicon



Barcode identifiers

While waiting for NGPD data…

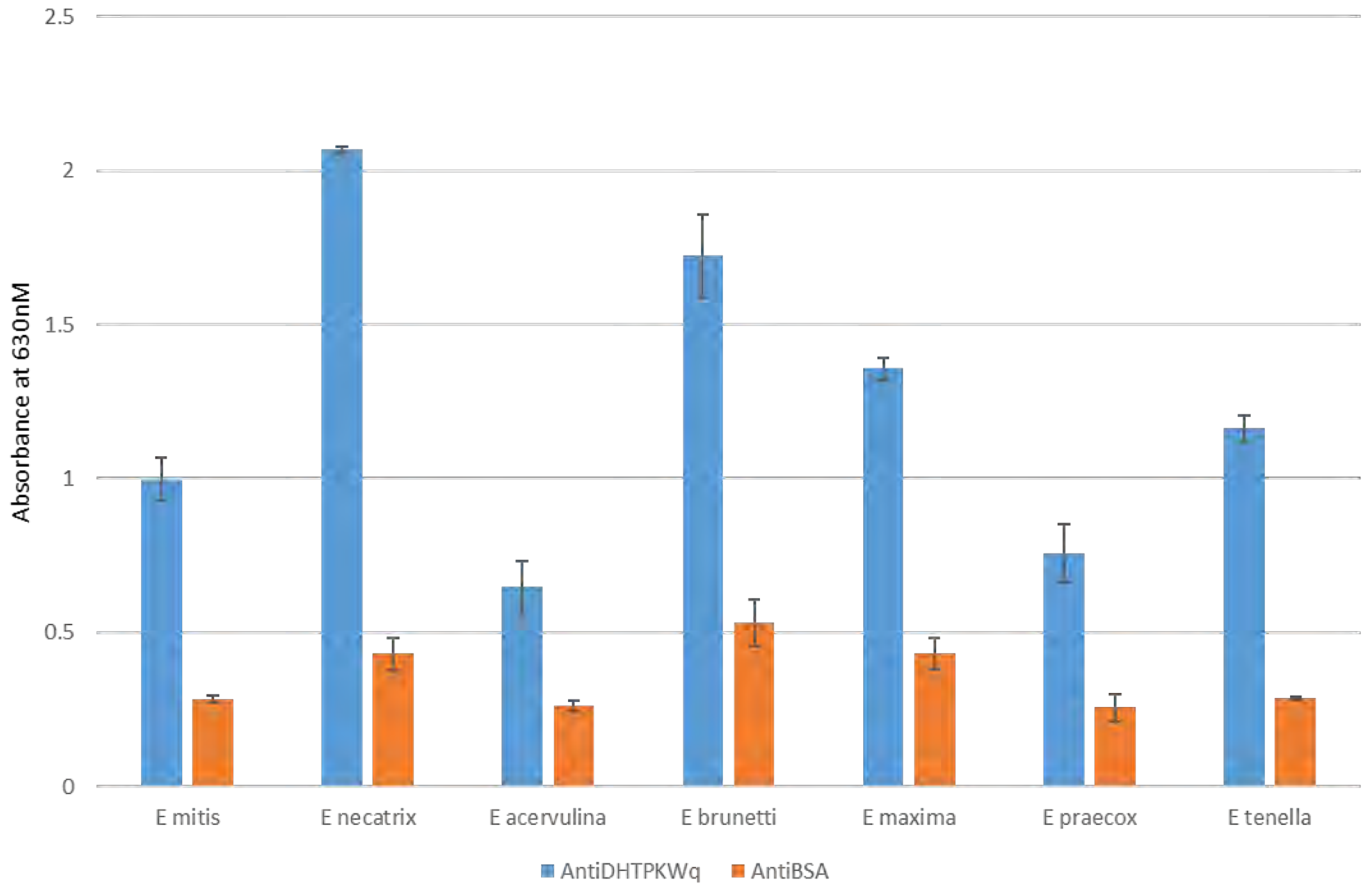
- 10 single clones were picked from each species *(round 5 panning)*
- Similar restriction enzyme digest pattern



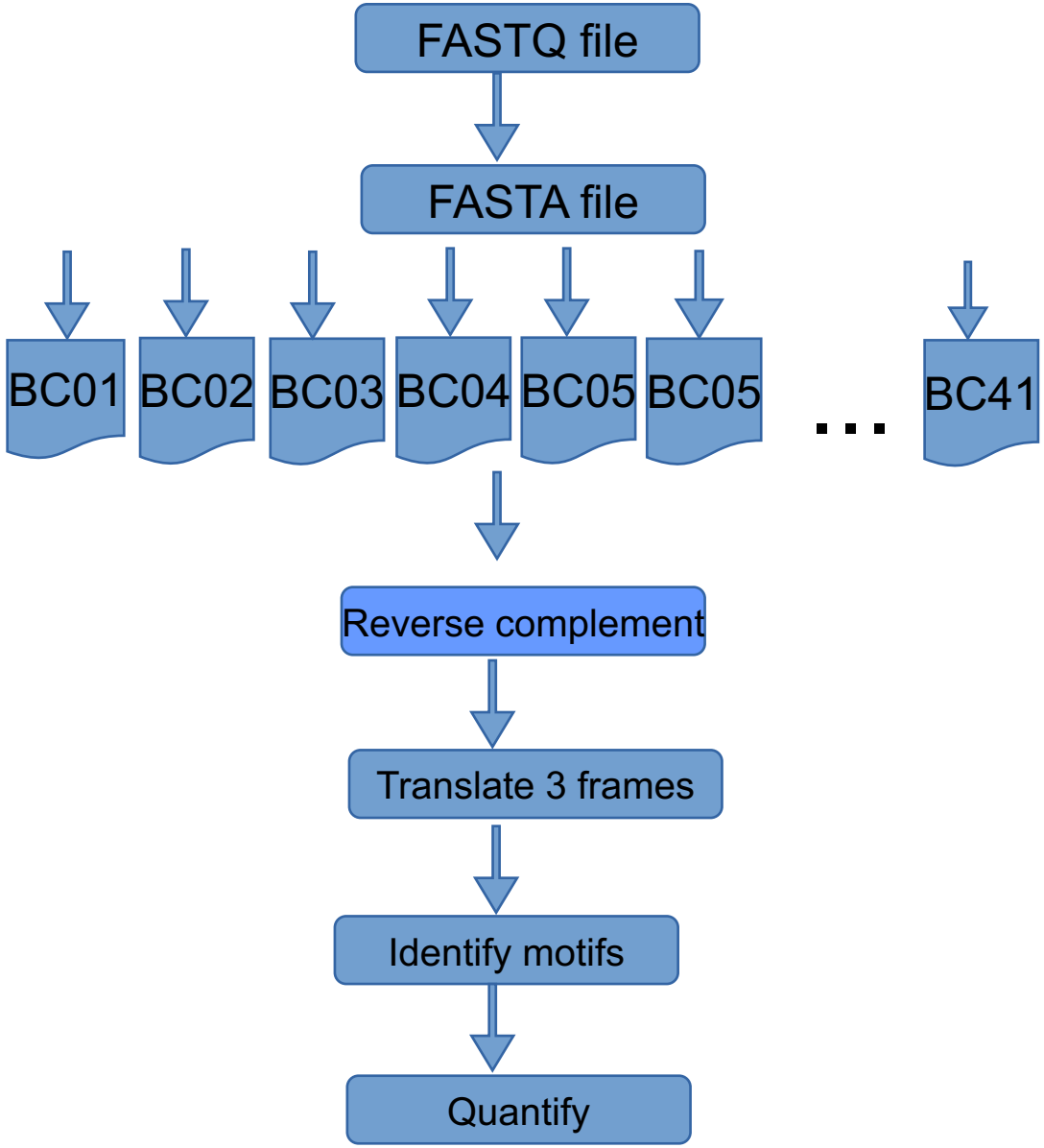
- 1/3rd (23 clones) randomly picked clones were Sanger sequenced DHTPKWq

- Screened via ELISA

Traditionally picked scFv DHTPKWq with AntiBSA phage ELISA



NGPD data analysis



Results

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Criteria for selection

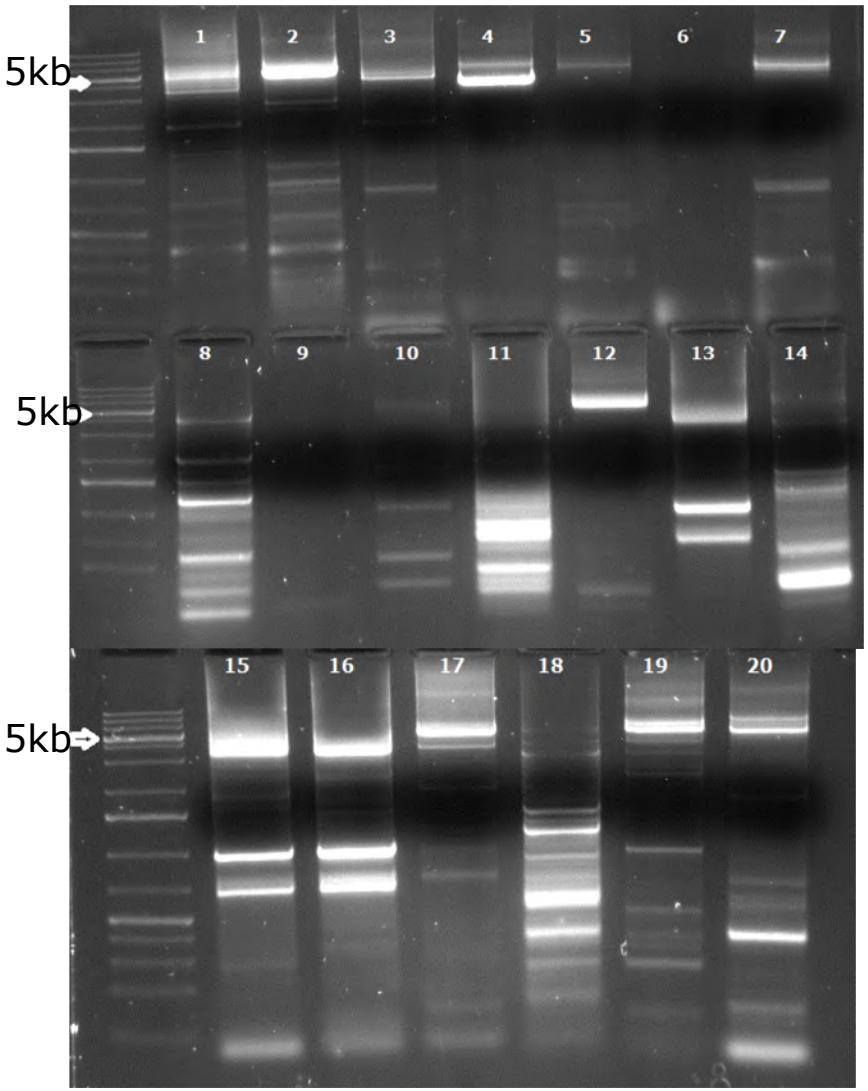
- Z scores (ratio and frequency of binding). They define relative enrichment
- Z score > 5
- At least 5 reads
- Enriched in at least 2 panning rounds
- Enriched in selection Phase not only growth

Selected scFv

1 AWTVNQTTNY
2 DGKPPSG
3 DRSYTACDTSK
4 DKSSqG
5 EDRqCKHG
6 ESSqMNS
7 VAADFDY
8 YIRYTSPV
9 STQHP
10 SSDAP
11 SPRLPFDY
12 QTGMN
13 LMVYKH
14 KDVGVVPK
15 IHRMVYL
16 HPMLGGLQGK
17 SRSKNYLWRSM
18 RDLTqDMWR
19 TTALP
20 SAqHP

From the DNA sequence, inverse PCR primers were designed to recover that phagemid from the panning round with the highest frequency of the clone

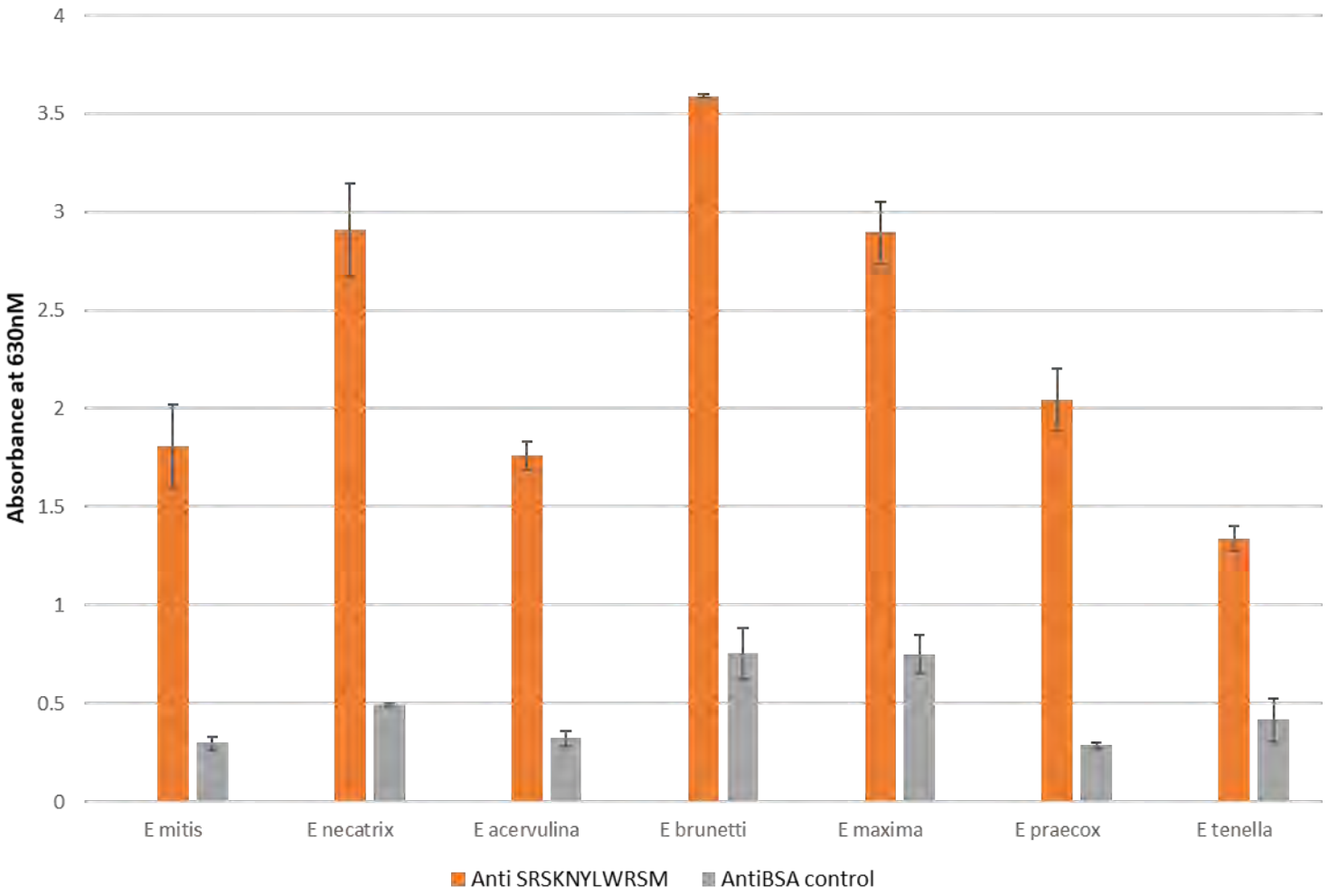
Inverse PCR (phagemid rescues)



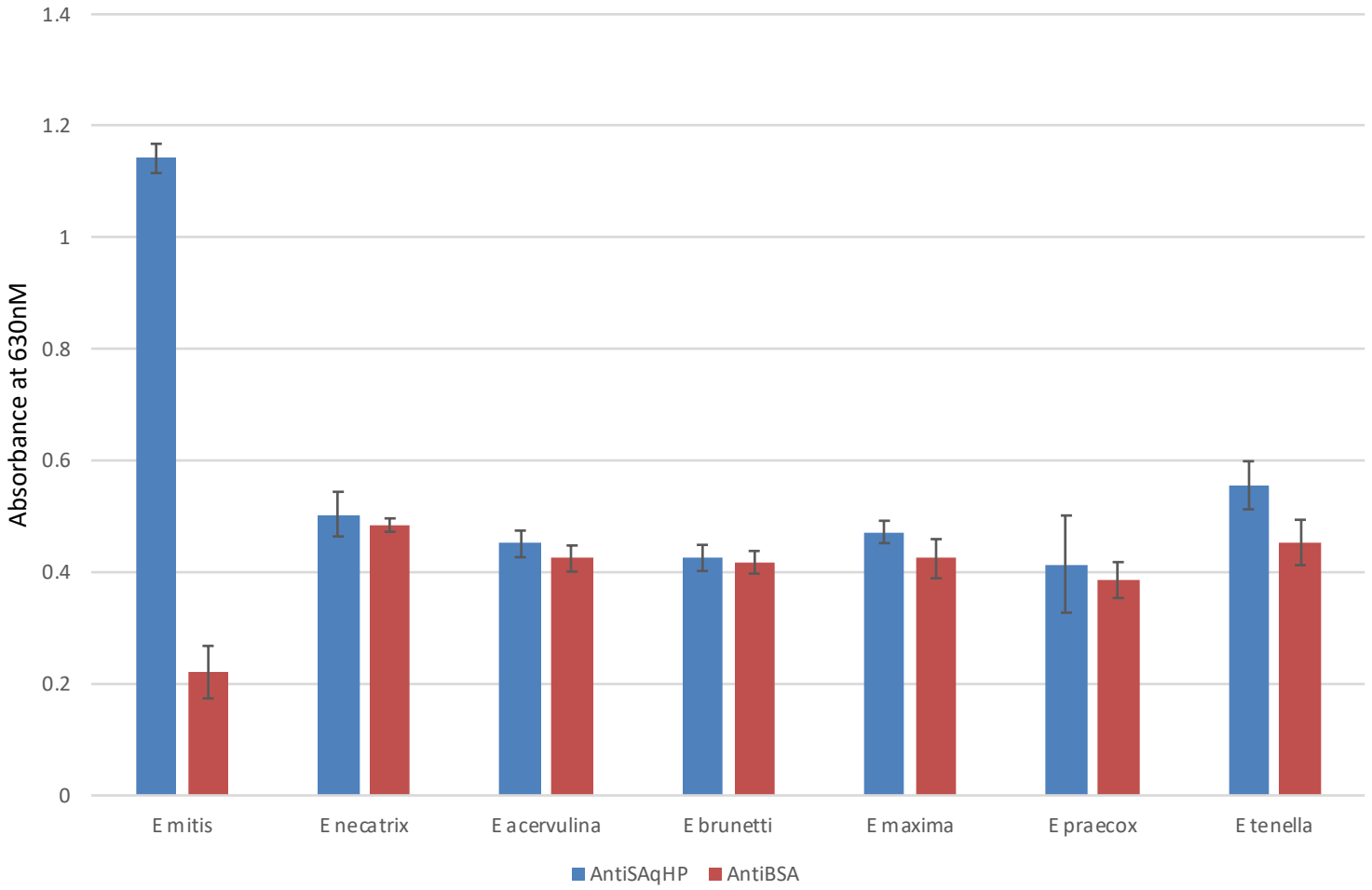
1 AWTVNQTTNY
2 DGKPPSG
3 DKSSqGT
4 DRSYTACDTSK
5 EDRqCKHG
6 ESSqMNS failed
7 VAADPFDY
8 YIRYTSPV
9 STQHP failed
10 SSDAP failed
11 SPRLPFDY failed
12 QTGMN
13 LMVYKH
14 KDVGVVPK failed
15 IHRMVYL
16 HPMLGGLQGK
17 SRSKNYLWRSM
18RDLTqDMWRfailed
19 TTALP
20 SAqHP

12 were recovered

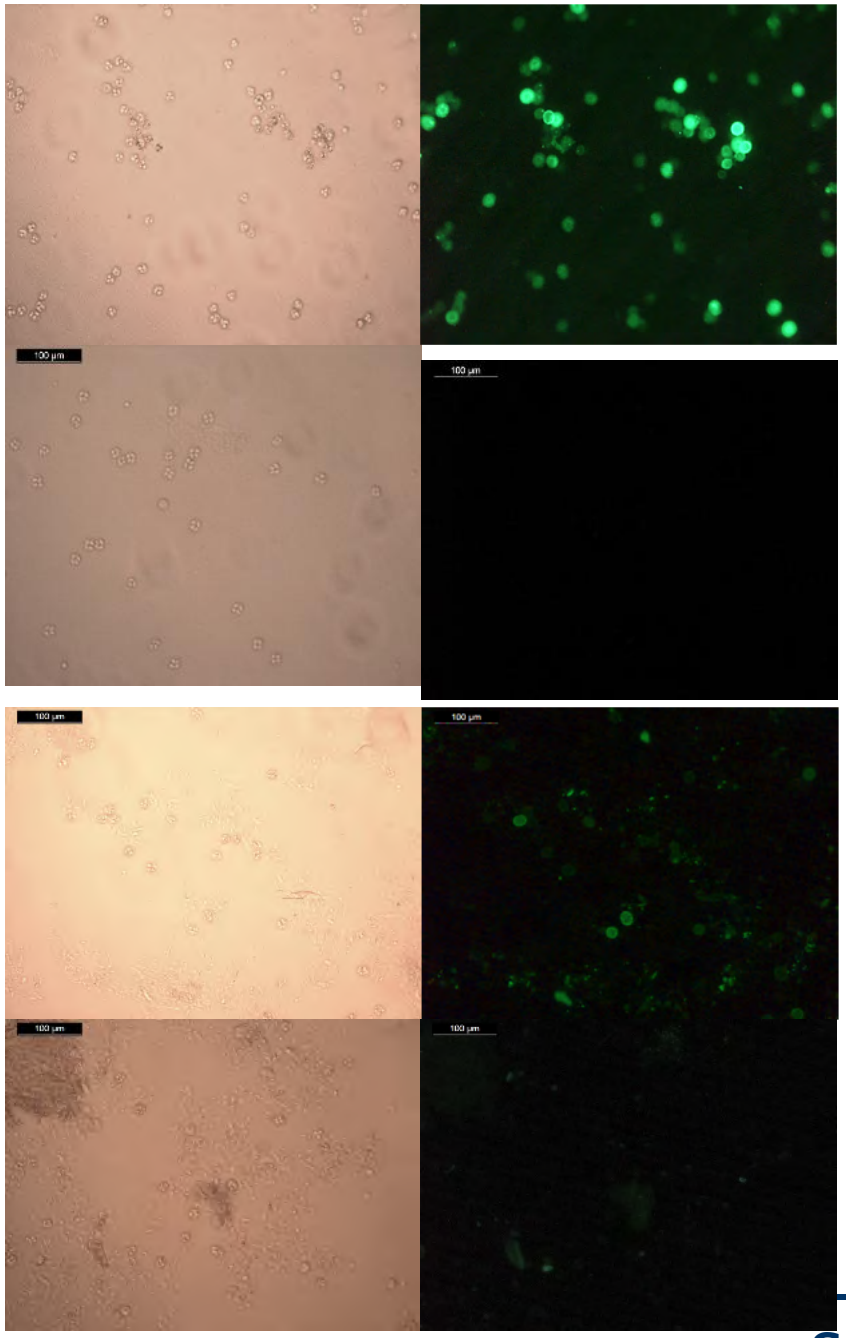
Rescued scFv AntiSRSKNYLWRSM with AntiBSA control phage ELISA



Rescued scFv AntiSAqHP with AntiBSA control phage ELISA

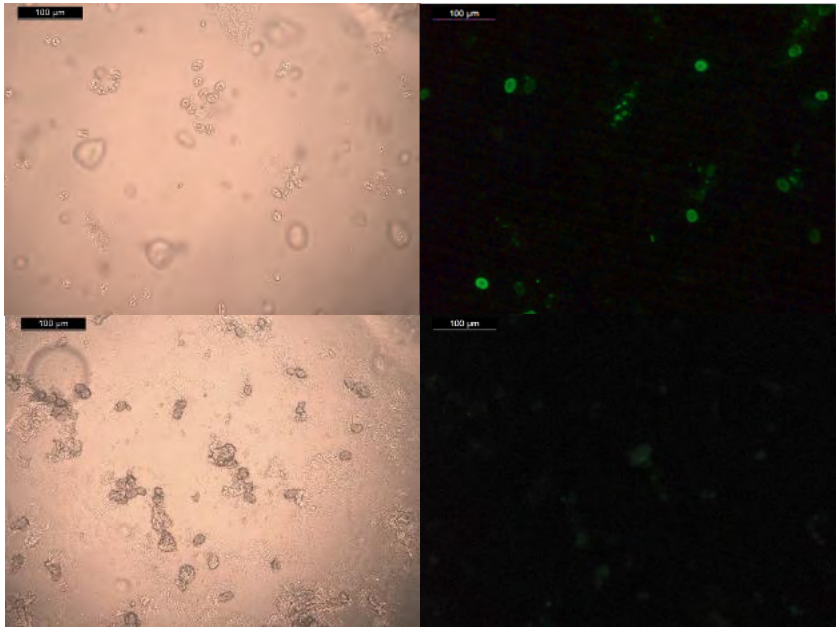


AntiSRSKNYLWRSM phage

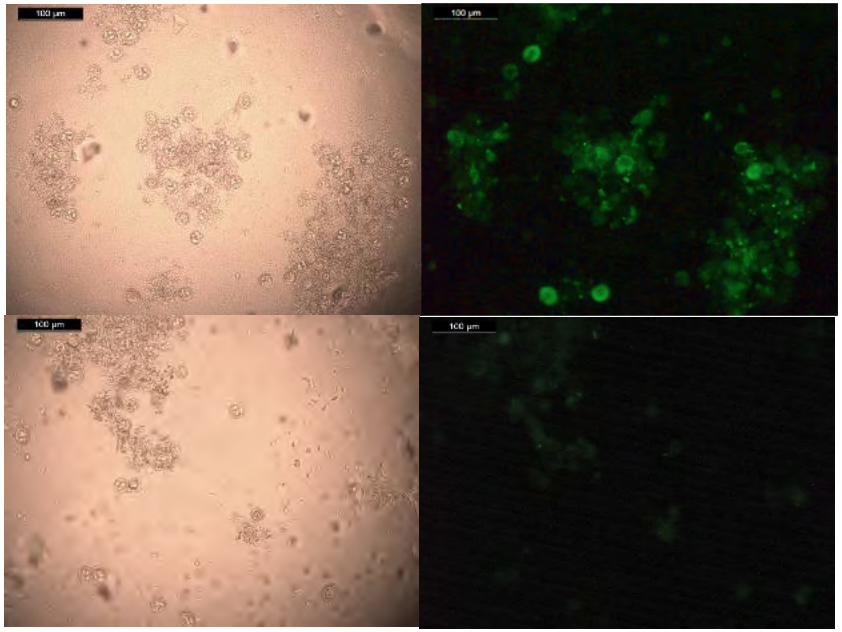


Eimeria mitis brightfield and fluorescent images above and BSA phage brightfield and fluorescent control below

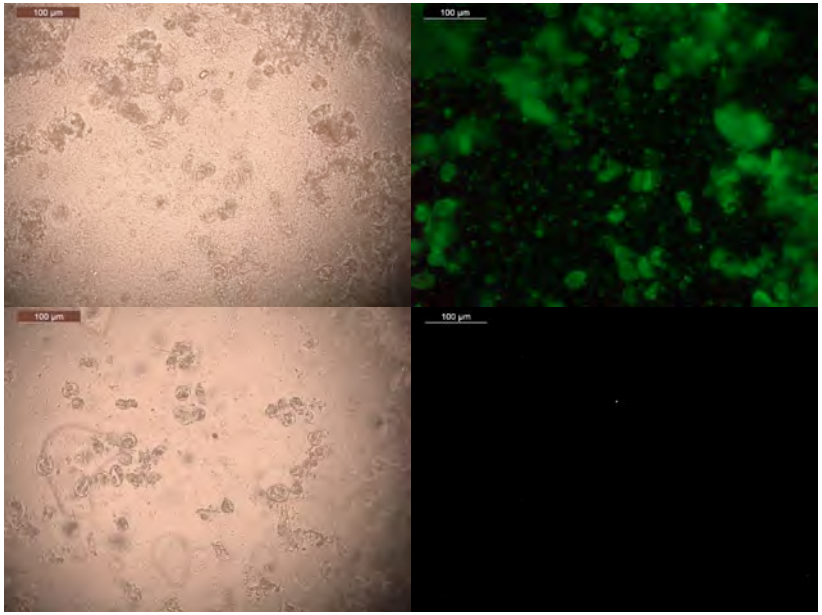
Eimeria necatrix brightfield and fluorescent images above and BSA phage brightfield and fluorescent control below



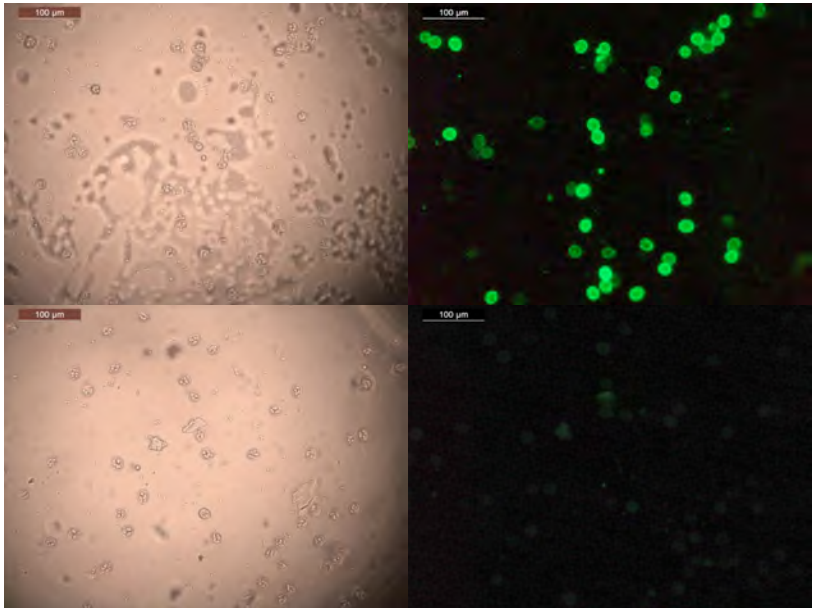
Eimeria acervulina brightfield and fluorescent images above and BSA phage brightfield and fluorescent control below



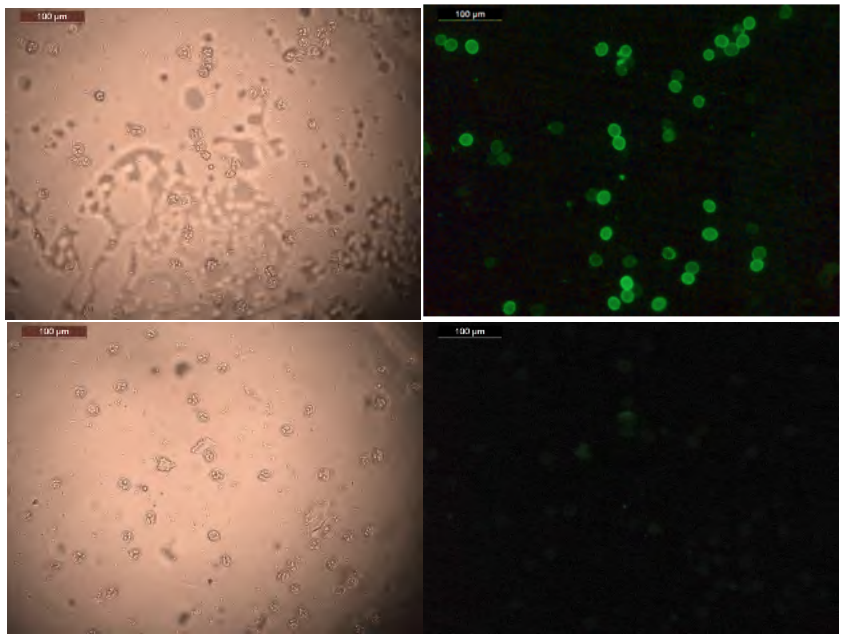
Eimeria brunetti brightfield and fluorescent images above and BSA phage brightfield and fluorescent control below



Eimeria maxima brightfield and fluorescent images above and BSA phage brightfield and fluorescent control below



Eimeria praecox brightfield and fluorescent images above and BSA phage brightfield and fluorescent control below



Eimeria tenella brightfield and fluorescent images above and BSA phage brightfield and fluorescent control below

Summary

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Objective 1
Isolate antibodies against oocysts of all seven *Eimeria* species

Objective 2
To compare traditional phage panning with Next generation phage display (NGPD)

- We isolated two antibodies against all seven *Eimeria* species
- We isolated an antibody against *Eimeria mitis*
- More antibodies are currently in development

- We found NGPD better because true binders can easily be missed by phage that have growth advantage

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