DEVELOPMENT OF RECOMBINANT HUMAN DIPHTHERIA ANTITOXIN

Diphtheria Antitoxin Monoclonal Antibody (DATMAB) Project

10th World Congress on Alternatives
and Animal Use in the Life Sciences

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Outline

- Equine antitoxin production
- Non-animal antitoxin production
- DATMAB project
- Early results

Equine antitoxin production

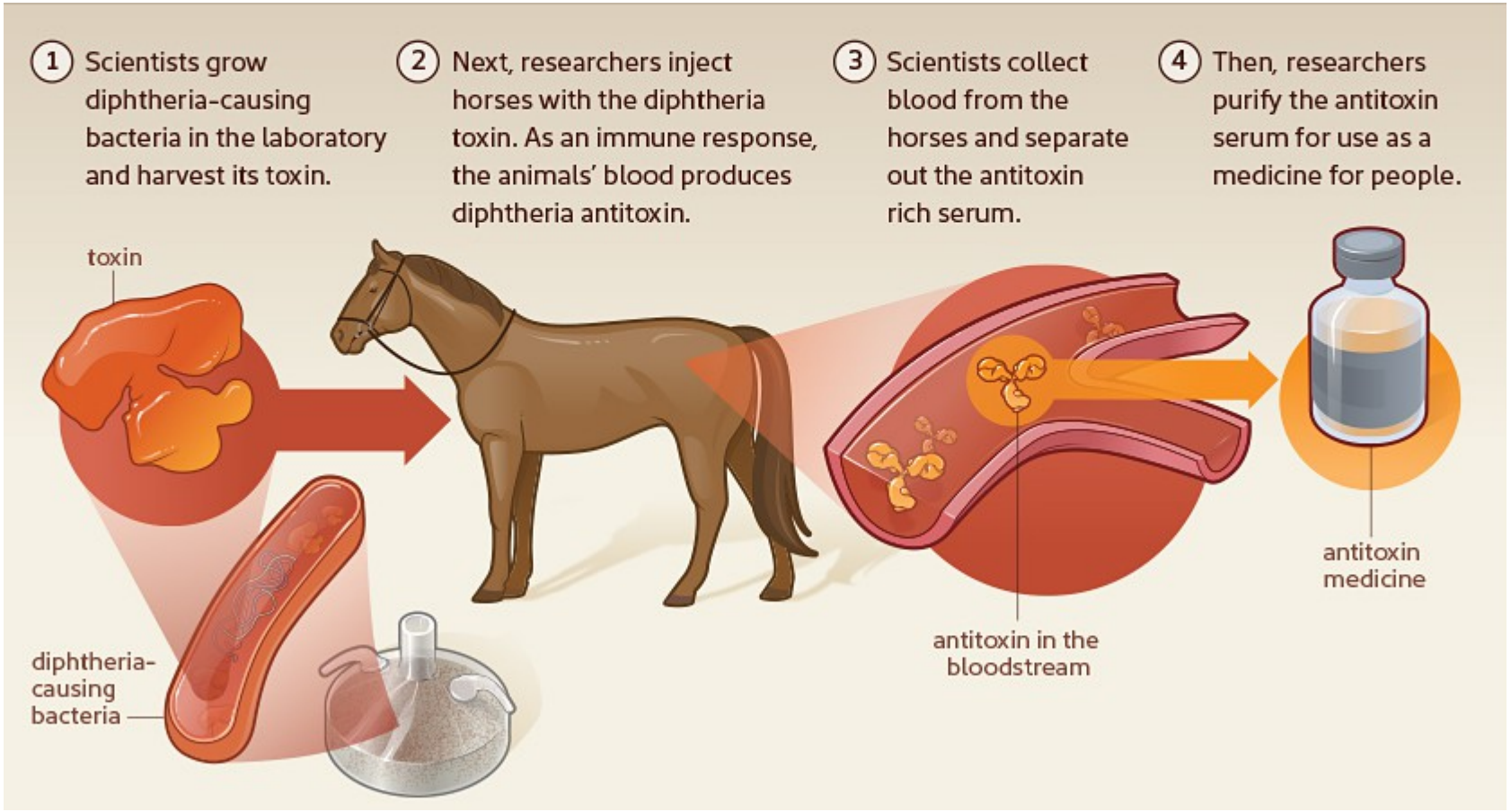


Image: https://www.nlm.nih.gov/exhibition/fromdnatobeer/exhibition-interactive/illustrations/diphtheria-alternative.html

Equine diphtheria antitoxin

- Front line treatment for diphtheria
- Inadequate supply
- Causes allergic reactions, serum sickness, etc.

Image: http://www.wpro.who.int/philippines/mediacentre/features/lessons_learned_diphtheria_cases/en/

Equine antitoxin production

Image: National Museum of American History

Equine antitoxin production in India

Images: PETA India

Recombinant antibodies on the market

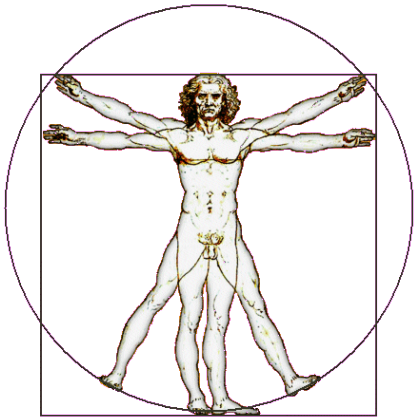
Product	Approved to treat	FDA licensure
Raxibacumab	Anthrax	2012
Adalimumab	Rheumatoid arthritis, Crohn's disease, ulcerative colitis, etc.	2002
Adalimumab-atto		2016
Belimumab (i.v.)	Systemic lupus erythematosus (SLE)	2011
Belimumab (self-inj.)		2017

DATMAB: Project summary

- Phage display to select recombinant human antibodies against diphtheria toxin
- Assess toxin neutralization of up to 15 toxin binding antibodies
- Select 2 neutralizing antibodies with best binding affinity, convert to IgG format
- Freeze-dry formulation and stability testing
- Public health stakeholders, further development

DATMAB: Phage display

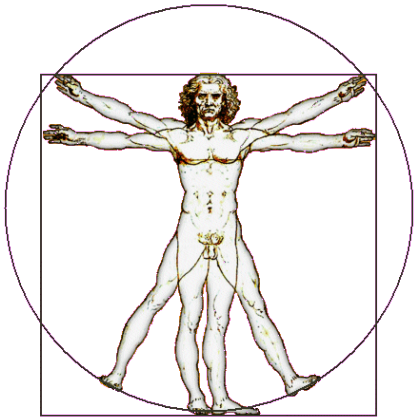
human



human
antibody gene
library

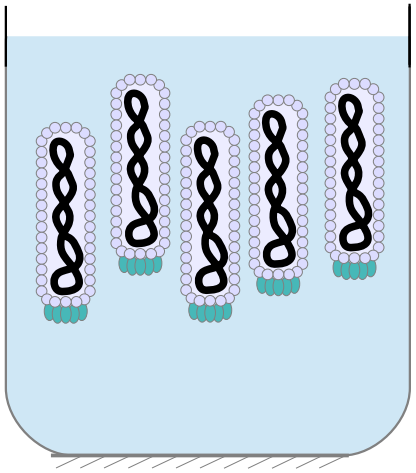
DATMAB: Phage display

human



human antibody gene library

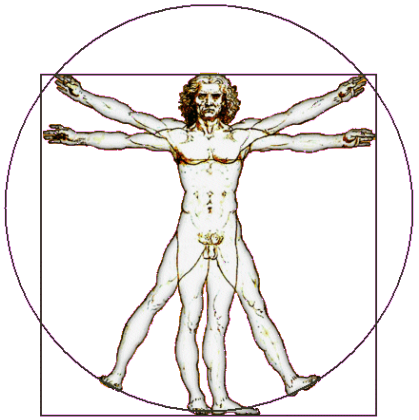
panning



selection of antibodies *in vitro* (panning)

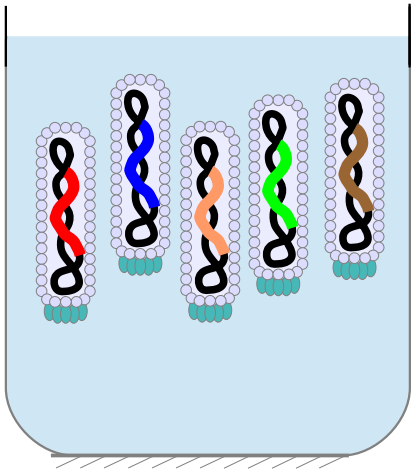
DATMAB: Phage display

human



human antibody gene library

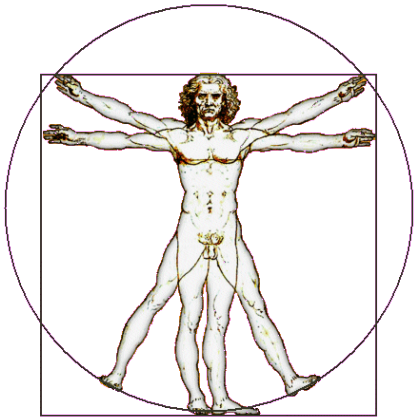
panning



selection of antibodies *in vitro* (panning)

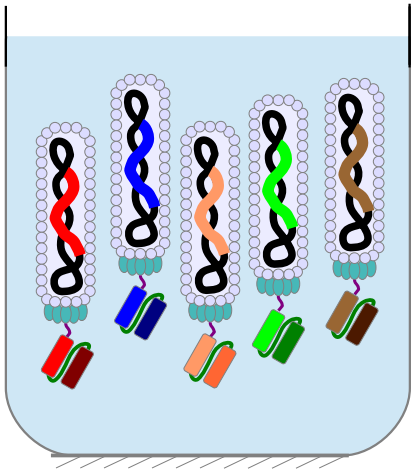
DATMAB: Phage display

human



human antibody gene library

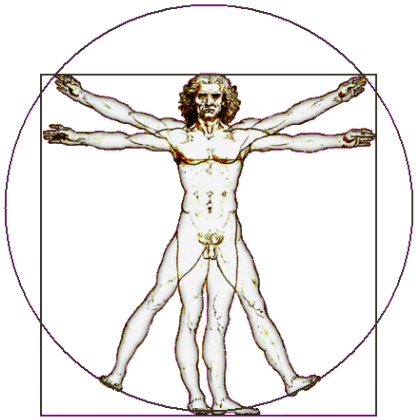
panning



selection of antibodies *in vitro* (panning)

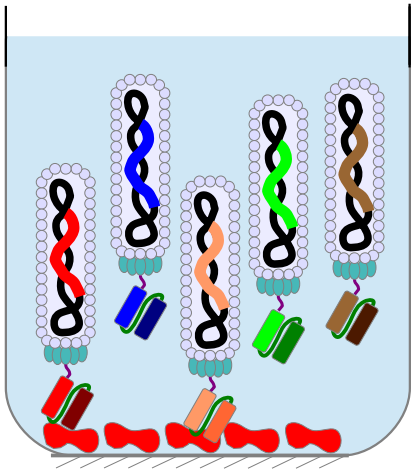
DATMAB: Phage display

human



human antibody gene library

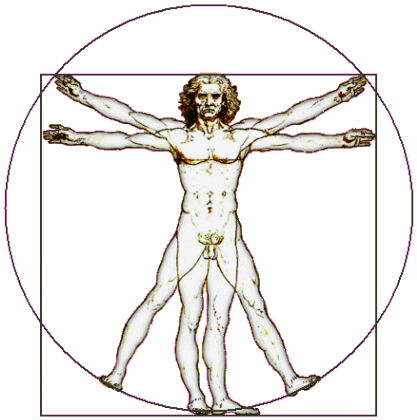
panning



selection of antibodies *in vitro* (panning)

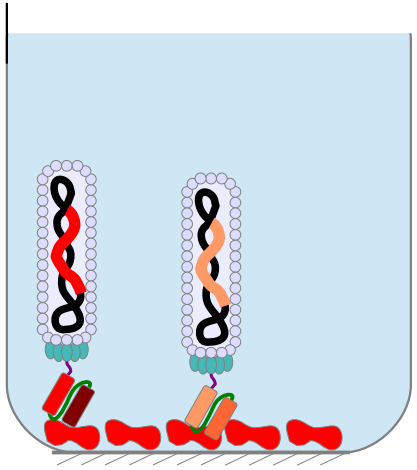
DATMAB: Phage display

human



human antibody gene library

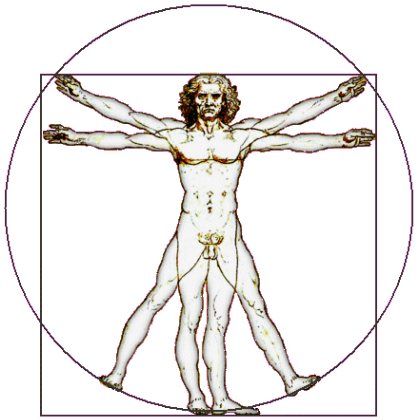
panning



selection of antibodies *in vitro* (panning)

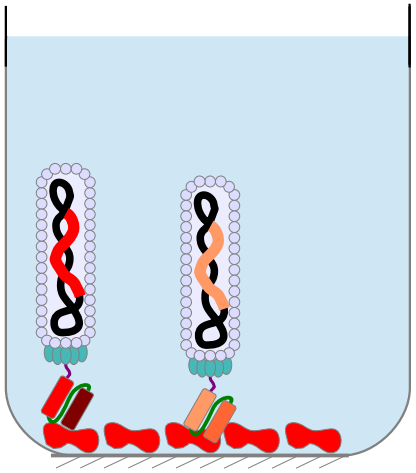
DATMAB: Phage display

human



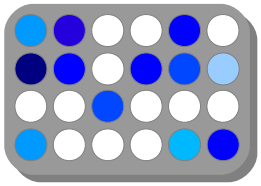
human antibody gene library

panning



selection of antibodies *in vitro* (panning)

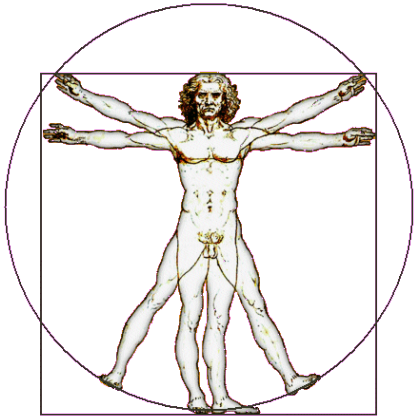
monoclonal antibodies



identification of monoclonal binders by ELISA

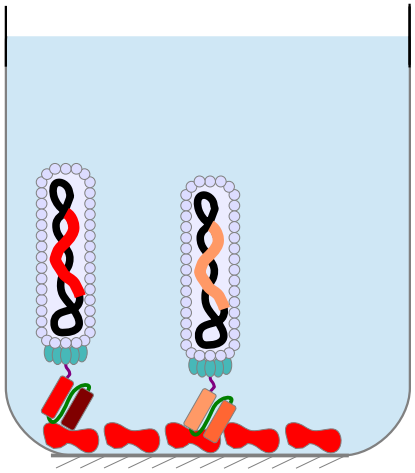
DATMAB: Phage display

human



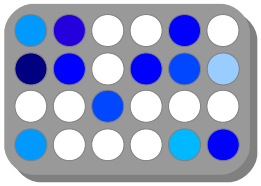
human antibody gene library

panning



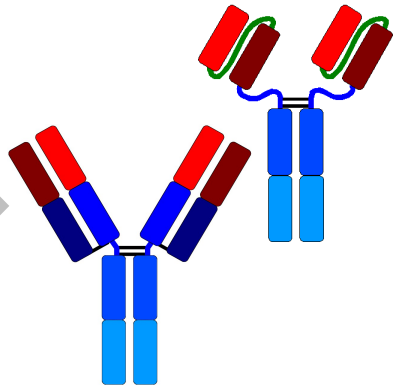
selection of antibodies *in vitro* (panning)

monoclonal antibodies



identification of monoclonal binders by ELISA

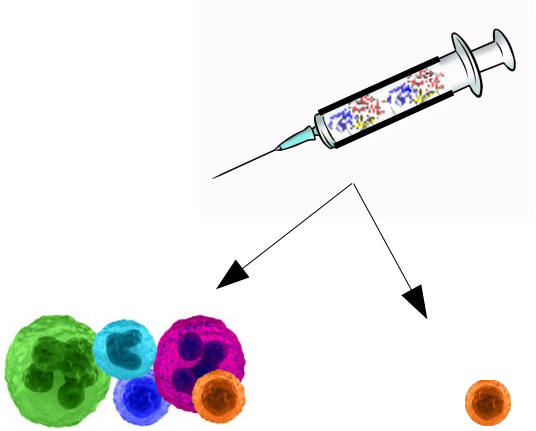
tailored antibodies



antibody engineering and production

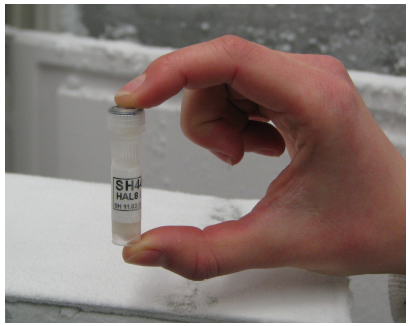
DATMAB: Phage display

Immune libraries



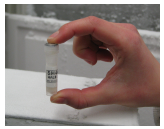
Total PBMC VJN & Sorted plasma cells CD138+

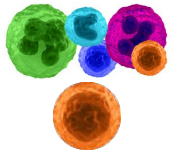
Naive library



HAL9 & HAL10

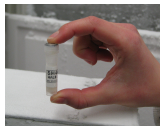
DATMAB: Phage display output

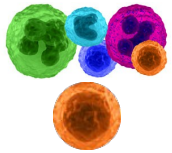




Library	Diphtheria toxin binding (scFv)	ELISA: unique sequences (scFv)
HAL9/ HAL10	39	27
VJN/ CD138+	132	100
Total	**171**	**127**

DATMAB: Phage display results





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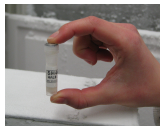
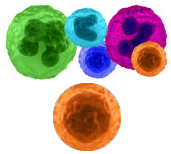
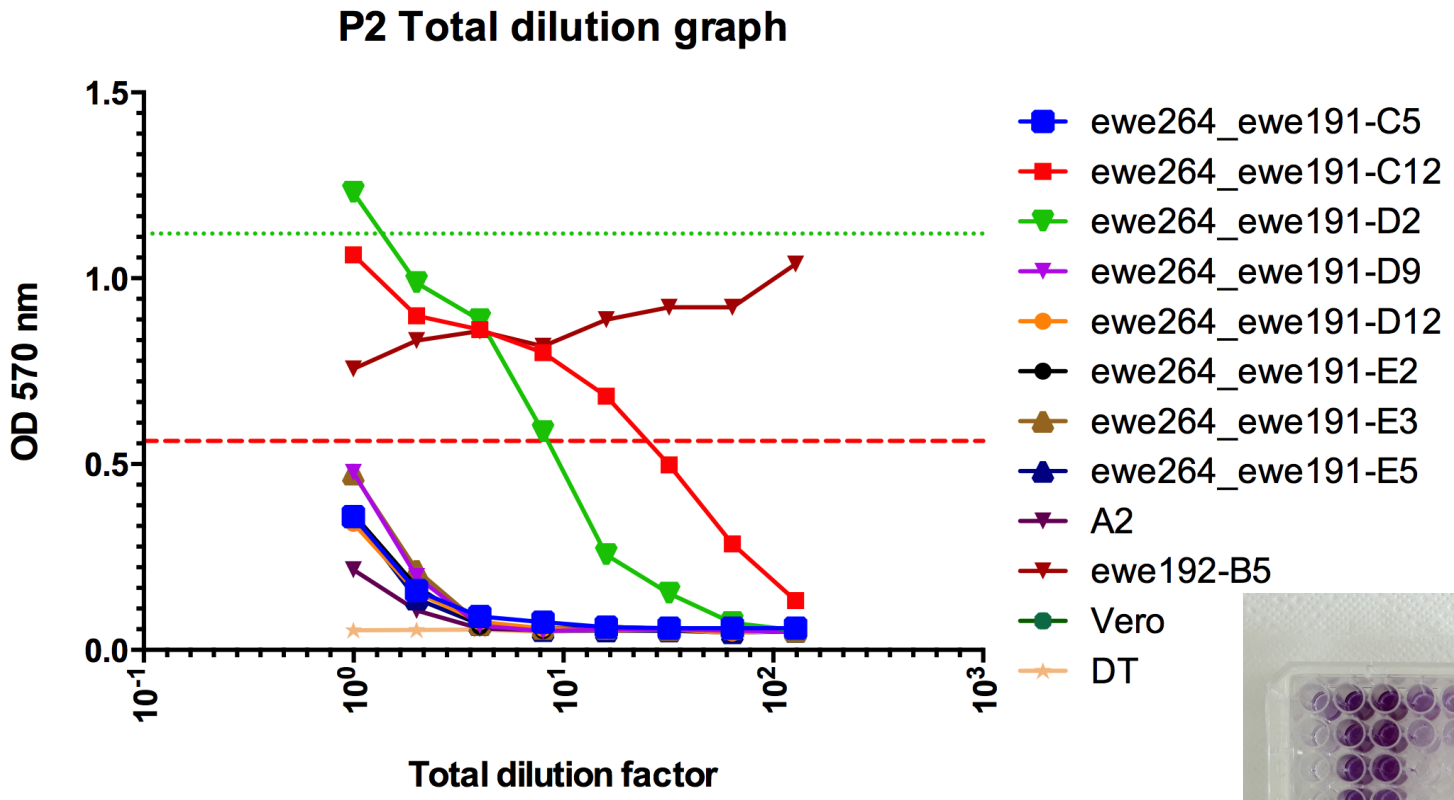
DATMAB: *In vitro* neutralization

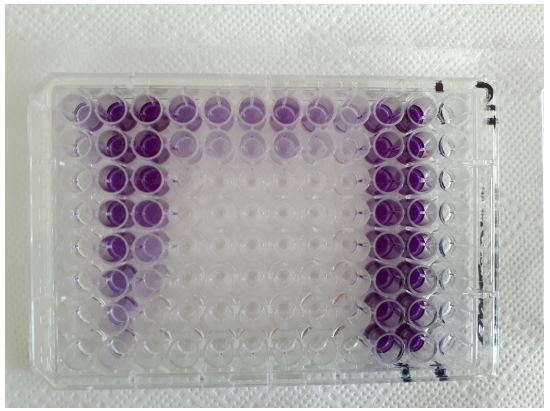




Library	ELISA: Unique sequences (scFv)	Vero cell TNT (scFv-Fc)
HAL9/ HAL10	27	0
VJN/ CD138+	100	25
Total	**127**	**25**

DATMAB: *In vitro* Vero cell TNT





DATMAB: Next steps

- Larger scale antibody production in mammalian cells
- Characterization (toxin binding, affinity, etc.)
- IgG cloning of best neutralizing antibodies
- Testing of antibody combinations
- Strategy for further regulatory development

Thank you

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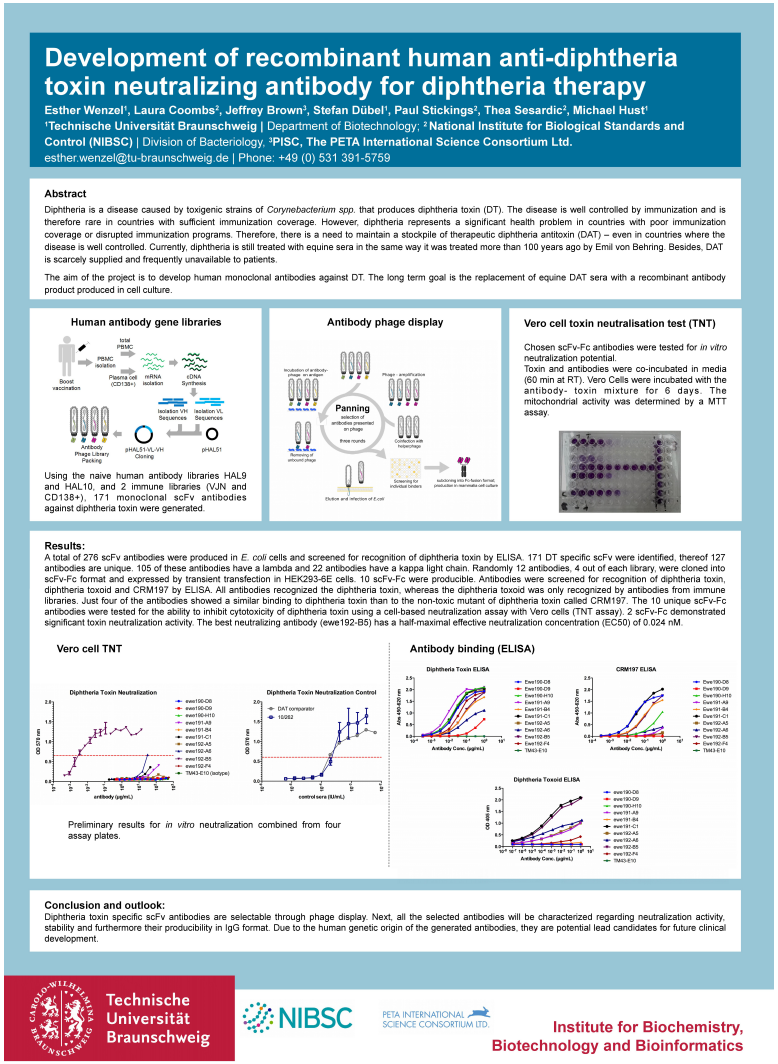
Technische Universität Braunschweig

CENTER FOR CONTEMPORARY EQUINE STUDIES



Development of recombinant human anti-diphtheria toxin neutralizing antibody for diphtheria therapy

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Abstract

Diphtheria is a disease caused by toxigenic strains of *Corynebacterium spp.* that produces diphtheria toxin (DT). The disease is well controlled by immunization and is therefore rare in countries with sufficient immunization coverage. However, diphtheria represents a significant health problem in countries with poor immunization coverage or disrupted immunization programs. Therefore, there is a need to maintain a stockpile of therapeutic diphtheria antitoxin (DAT) – even in countries where the disease is well controlled. Currently, diphtheria is still treated with equine sera in the same way it was treated more than 100 years ago by Emil von Behring. Besides, DAT is scarcely supplied and frequently unavailable to patients.

The aim of the project is to develop human monoclonal antibodies against DT. The long term goal is the replacement of equine DAT sera with a recombinant antibody product produced in cell culture.

Human antibody gene libraries

Using the naive human antibody libraries HAL9 and HAL10, and 2 immune libraries (VJN and CD138+), 171 monoclonal scFv antibodies against diphtheria toxin were generated.

Antibody phage display

Vero cell toxin neutralisation test (TNT)

Chosen scFv-Fc antibodies were tested for *in vitro* neutralization potential.
Toxin and antibodies were co-incubated in media (60 min at RT). Vero Cells were incubated with the antibody- toxin mixture for 6 days. The mitochondrial activity was determined by a MTT assay.

Results:

A total of 276 scFv antibodies were produced in *E. coli* cells and screened for recognition of diphtheria toxin by ELISA. 171 DT specific scFv were identified, thereof 127 antibodies are unique. 105 of these antibodies have a lambda and 22 antibodies have a kappa light chain. Randomly 12 antibodies, 4 out of each library, were cloned into scFv-Fc format and expressed by transient transfection in HEK293-6E cells. 10 scFv-Fc were producible. Antibodies were screened for recognition of diphtheria toxin, diphtheria toxoid and CRM197 by ELISA. All antibodies recognized the diphtheria toxin, whereas the diphtheria toxoid was only recognized by antibodies from immune libraries. Just four of the antibodies showed a similar binding to diphtheria toxin than to the non-toxic mutant of diphtheria toxin called CRM197. The 10 unique scFv-Fc antibodies were tested for the ability to inhibit cytotoxicity of diphtheria toxin using a cell-based neutralization assay with Vero cells (TNT assay). 2 scFv-Fc demonstrated significant toxin neutralization activity. The best neutralizing antibody (ewe192-B5) has a half-maximal effective neutralization concentration (EC50) of 0.024 nM.

Vero cell TNT

Preliminary results for *in vitro* neutralization combined from four assay plates.

Antibody binding (ELISA)

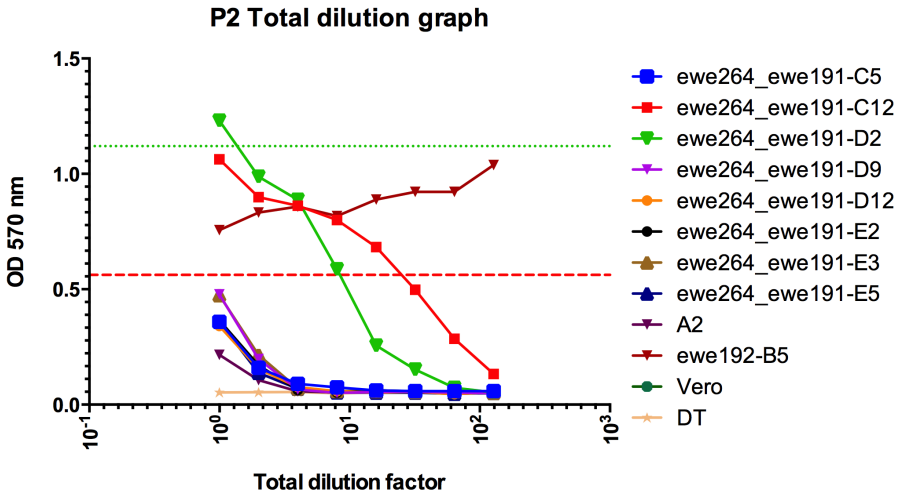
Conclusion and outlook:

Diphtheria toxin specific scFv antibodies are selectable through phage display. Next, all the selected antibodies will be characterized regarding neutralization activity, stability and furthermore their producibility in IgG format. Due to the human genetic origin of the generated antibodies, they are potential lead candidates for future clinical development.

Technische Universität Braunschweig | NIBSC | PETA INTERNATIONAL SCIENCE CONSORTIUM LTD. | Institute for Biochemistry, Biotechnology and Bioinformatics

DATMAB Diphtheria toxin neutralization

- detection by MTT Assay

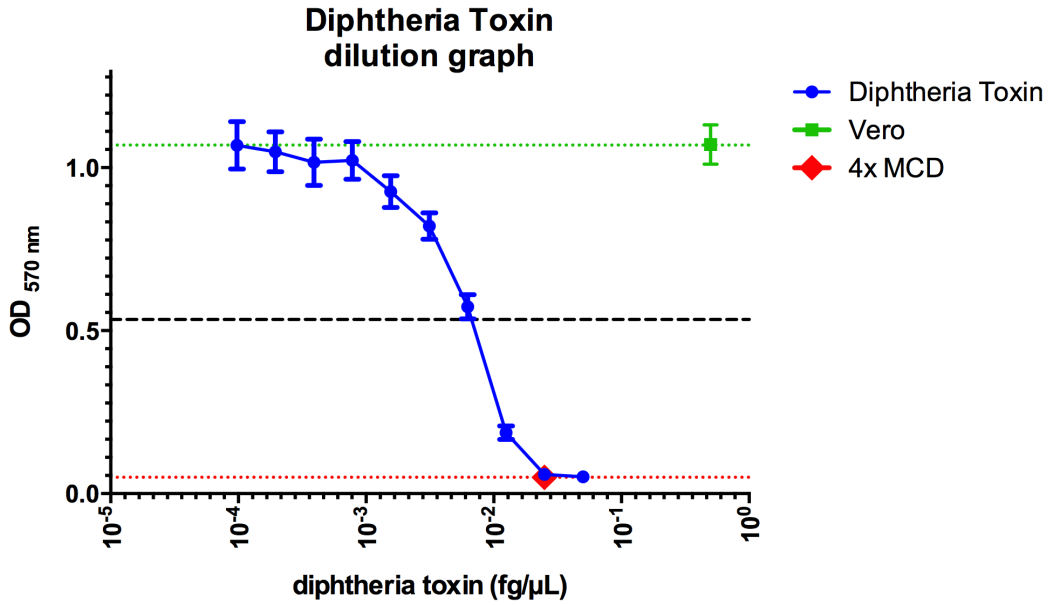


- Testing of 25 purified scFv-Fc antibodies for neutralization of diphtheria toxin

DATMAB Vero cell assay titration

- Diphtheria toxin (List biological labratories, inc, #150, Lot 15043A1)

  detection by MTT Assay



Recombinant antibodies

Diphtheria

- Caused by *Corynebacterium* toxigenic strains, vaccine preventable
- 5-10% cases fatal (20% for <5 years and >40 years)
- 102 cases reported 2009-2012 in the EU
- Equine diphtheria antitoxin (DAT) frontline treatment for new cases

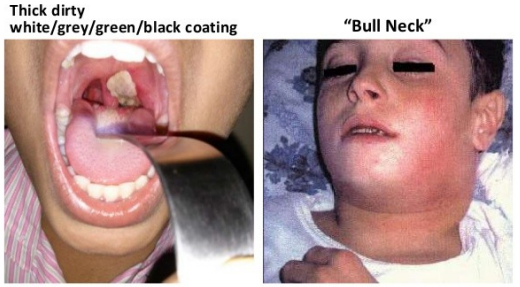


Fig: Diphtheria (slideshare.net)

Fig: DAT (wpro.who.int)

Equine antitoxin production

Equine antitoxin production

- Single polypeptide chain of 535 amino acids (M_r 58 kDa)
- fragment A (M_r 21 kDa), fragment B (M_r 37 kDa)
- 3 structural/functional domains:
  - **catalytic domain** (N-terminal ADP-ribosyltransferase)
  - **transmembrane domain** (region which facilitates the delivery of the catalytic domain across the cell membrane)
  - the eukaryotic cell **receptor binding domain**