

Research on and generation of a decavalent equine antitoxin counteracting deliberate botulinum neurotoxin attacks

X-BAT

anr[©]
agence nationale
de la recherche

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Contact : Lemichez

COORDINATEUR : Emmanuel LEMICHEZ

PARTENAIRES : Inst. Cochin, IRBA, MHH, WDT

Résumé :

BoNT neurotoxins from Clostridium botulinum represent a rising field of research in terms of medical but still is a public health issue in terms of food surveillance and risk of misuse as biological weapons. Within a Franco-German consortium, we develop a new pipeline of antitoxin production covering major BoNT serotypes as well as cutting edge cell biology methods of antitoxins qualification avoiding animal experiments.

CONTEXTE ET OBJECTIFS

Botulinum neurotoxins (BoNT) are known as the most toxic agents for humans and have been intended to be used as bio-weapons in military conflicts or terroristic attacks. The only proven therapy post-BoNT intoxication is the administration of BoNT serotype-specific antitoxin. The objective of the X-BAT consortium is to deliver

- 1) Highly available, EU-licensable medicine upon deliberate BoNT intoxications,
- 2) antitoxin coverage against all ten known BoNT serotypes employing innovative antigens and animal protecting routes of production
- 3) Research and implementation of animal replacement methods

MÉTHODOLOGIE ET RÉSULTATS

A novel pipeline for BoNT/A toxoid production, characterization, horse immunization and serum /F(ab')₂ production, anti-BoNT titer F(ab')₂ characterization and cell-based assay to measure BoNT activity.

Botulinum neurotoxins BoNT toxoids A,B, E and (F) produced and characterized (the serotypes responsible of a majority of human intoxications).

Horse immunization with validated Ig-based immune anti-BoNT/A responses?

Pipeline for anti-BoNT titer determination according to European Pharmacopae established for anti-BoNT/A.



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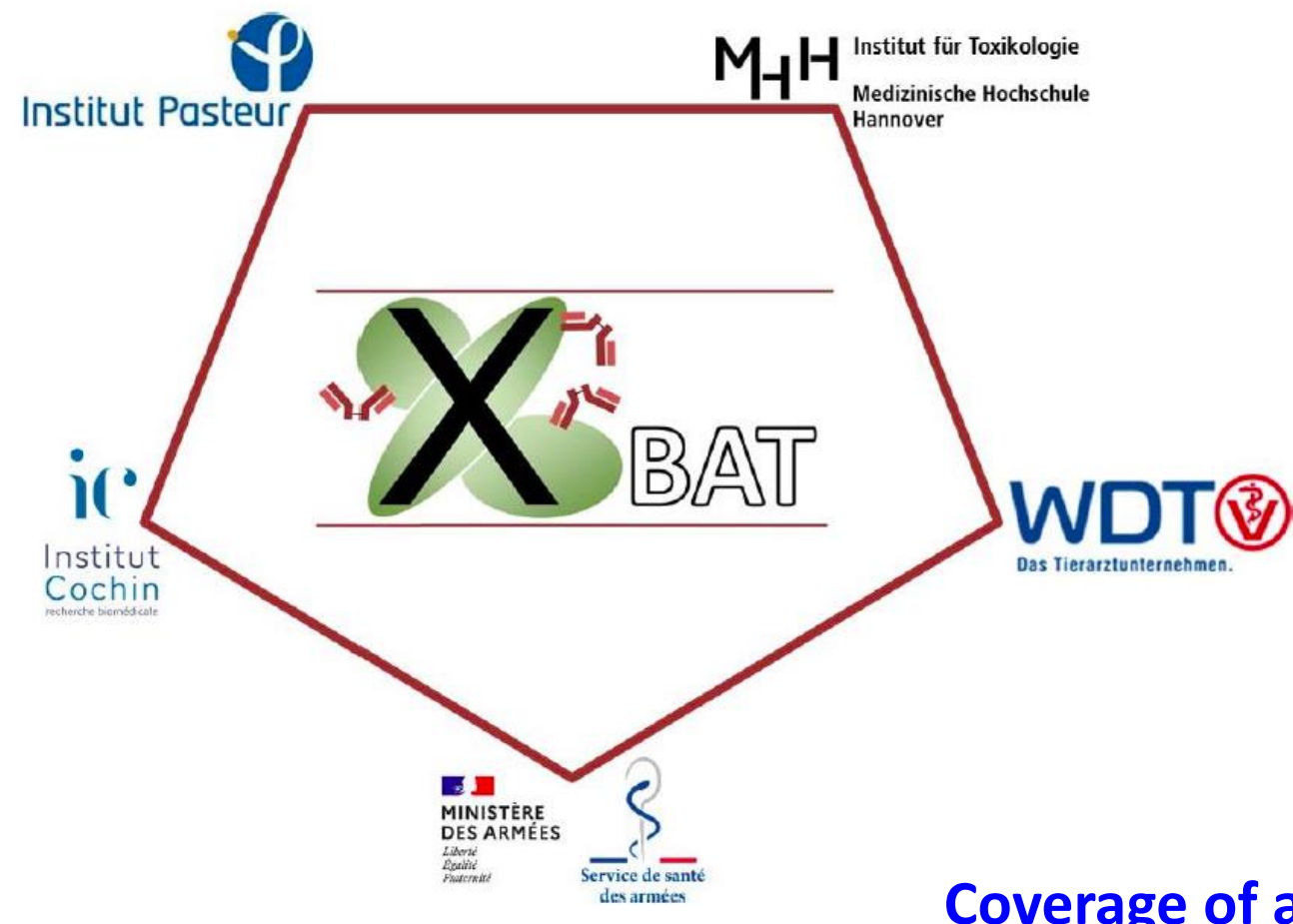
Simone Ladel¹, Maud Marechal², Marie-Anne Nahori², Christine Rasetti-Escargueil², Mona Engelke¹, Thomas Buchholz¹, Carmen Klepka³, Arnaud Avril⁴, Evelyne Bloch-Gallego⁵, Olaf Assenheimer¹, Andreas Rummel^{3,*} and **Emmanuel Lemichez^{2,*}**

1) WDT, Garbsen; 2) Institut Pasteur, Paris; 3) IRBA, Bretigny-sur-Orge; 4) Institut Cochin, Paris; 5) MHH and Toxigenics, Hannover; *) coordination



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X-BAT French-German consortium

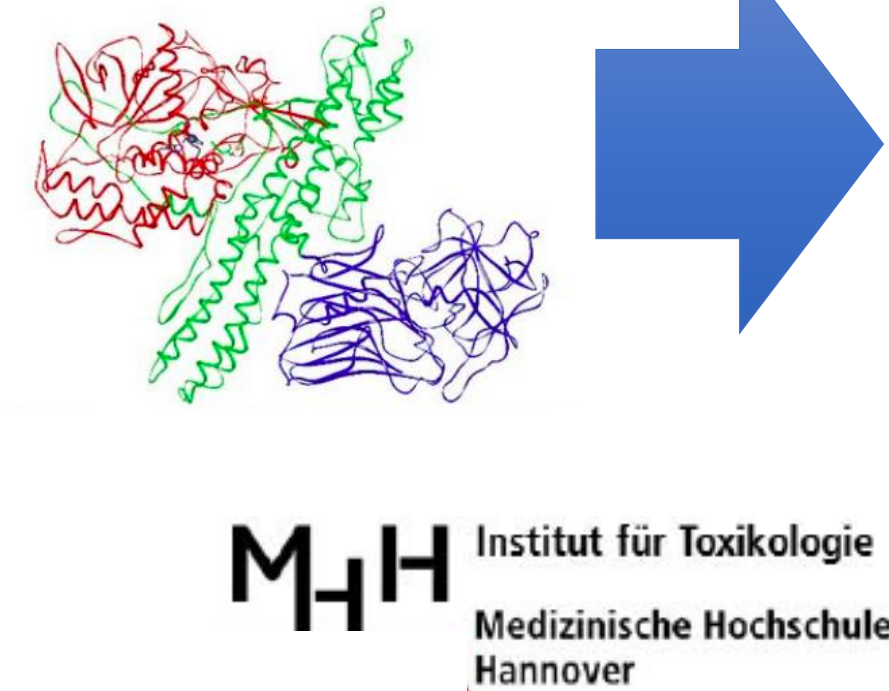


- Production of recombinant BoNT serotypes wildtype and toxoid
- Horse immunization and serum to F(ab')₂ production
- Toxin activity and serum anti-BoNT titer determination
- Development of a cell-based assay to measure BoNT activity and reduce
- Animal handling and suffering (3R rules)

Coverage of all know-how in

A novel pipeline for BoNT/A toxoid production (1), characterization (2), horse immunization and serum /F(ab')₂ production (3), anti-BoNT titer F(ab')₂ characterization (4) and cell-based assay to measure BoNT activity (5)

(1) BoNTi design & production



(2) Neurotoxin & toxoids activity determination

(March_May 2021)
BoNT/A MILD₅₀ ≈ 11pg (EURM111 reference material)

BoNT/Ai MILD₅₀ ≈ 5.7 µg
BoNT/Bi MILD₅₀ ≈ 0.36 µg
BoNT/Ei MILD₅₀ ≈ 27 µg



BoNT/Ai-Link MILD₅₀ residual activity

(4)

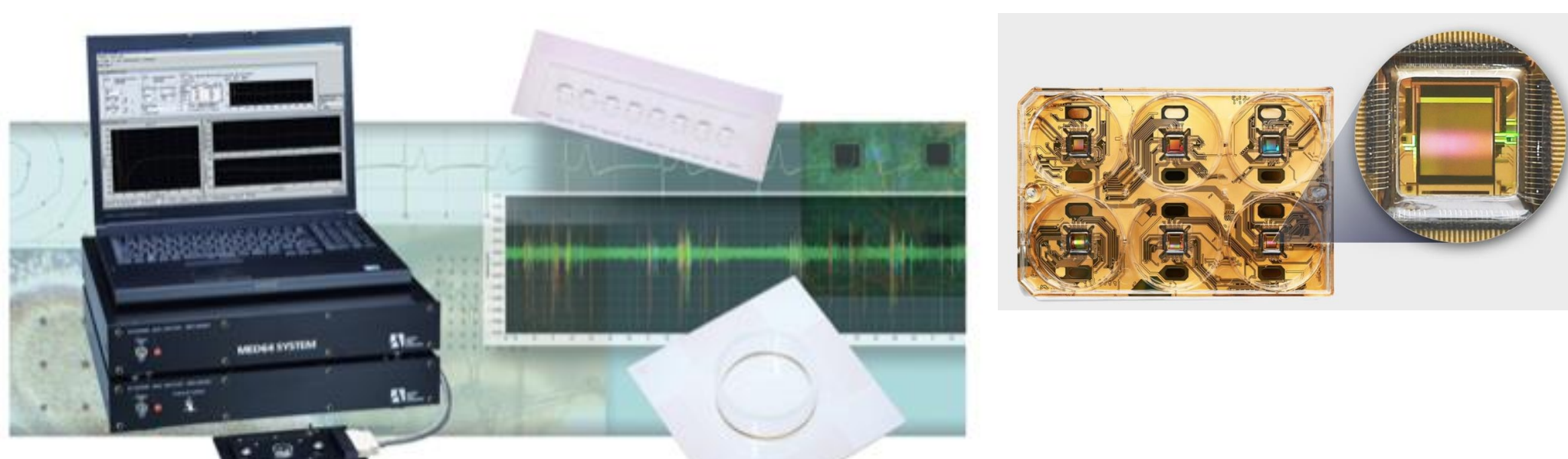
F(ab')₂ anti-BoNT protective titre: European pharmacopea 01/2020:0085



Mouse model
BoNT/A1 EURM111 reference material
BlutBang 05.04-12.04.18
Horse sera (319/338/343/349)

(5)

iPSC-based recording of botulinum neurotoxin activities and serum titer

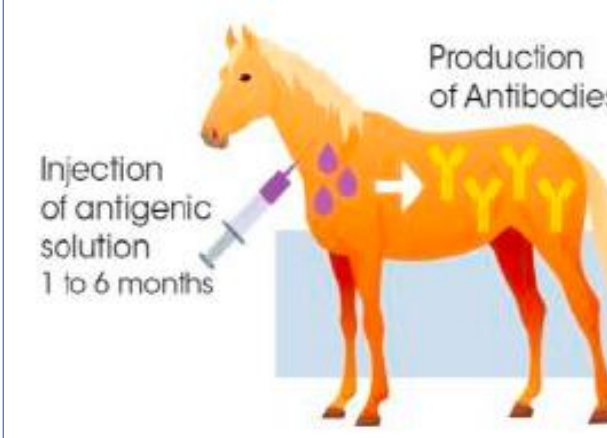


Equipment acquired : IP/ANR (December 2021)



(3)

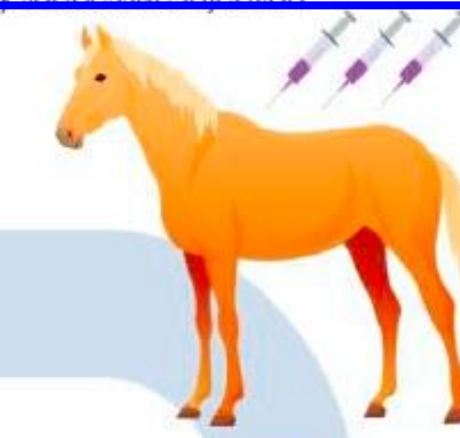
Hyperimmunization



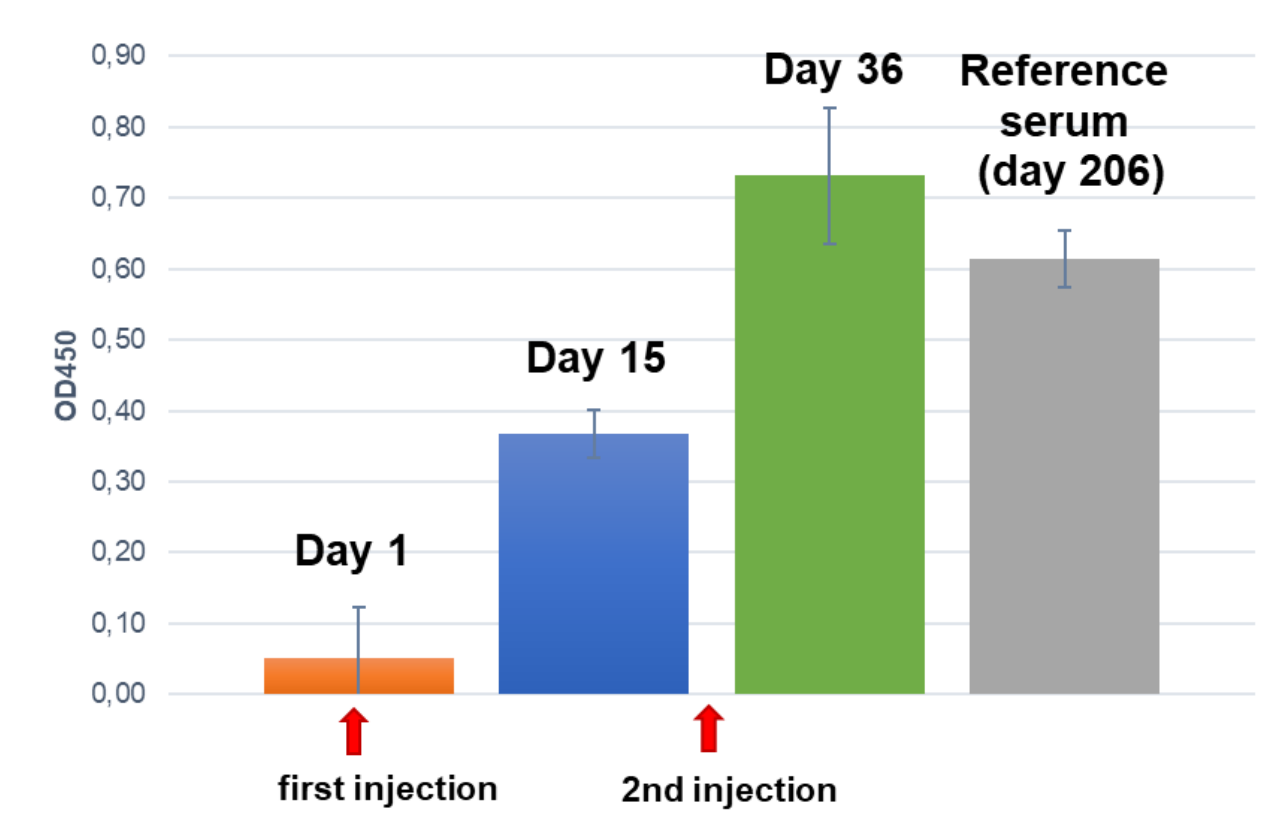
Production of Antibodies
Injection of antigenic solution 1 to 6 months

BoNT/A (July 2021)
BoNT/B (November 2021)
BoNT/E (March 2022)
BoNT/F (March 2022)

F(ab')₂ Production and Purification



Signal development BoNT/Ai-Link



anti-BoNT/A antibody level

Results and conclusions

anti-BoNT/A,B,E, F prototype under development

- ✓ Botulinum neurotoxins BoNT toxoids A,B, E and (F) produced and characterized (the serotypes responsible of a majority of human intoxications)
- ✓ Horse immunization with validated Ig-based immune anti-BoNT/A responses
- ✓ Pipeline for anti-BoNT titer determination according to European Pharmacopae established for anti-BoNT/A