Challenges in the replacement of *in-vivo* testing for Clostridial vaccines

29 Sept 2015

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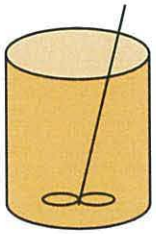
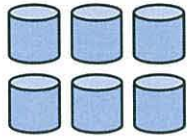
Introduction

- There are two key drivers behind vaccine testing:
  - Regulatory requirements to ensure safety and efficacy of released product. (APVMA, European Pharmacopoeia)
  - Process requirements to ensure product is formulated to meet release requirements and that the finished product meets the registered release specification.

Antigen Production Process

- *C. novyii*
- *C. septicum*
- *C. perfringens D*
- *C. chauveoi*
- *C. tetanii*
- *C. botulinum C/D*





Start — **Released Antigen**

Culture growth | **Inactivation** | **Processing** | **Testing**

0 — **2** — **4** — **6** — **9 Weeks**

Antigen Potency -

Before Inactivation

L+ Test

Demonstrates that the level of toxin is sufficient prior to further processing (LD_{50})

Residual Toxicity

Demonstrates that the toxins are inactivated

Antigen Potency -

Post Inactivation

Total Combining Power Test

Quantifies the toxoid (inactivated toxin) to provide levels for formulation purposes

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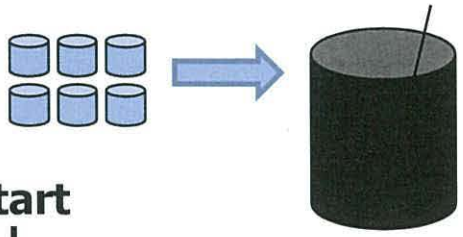
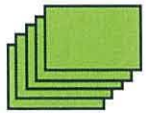
Vaccine Manufacturing Process

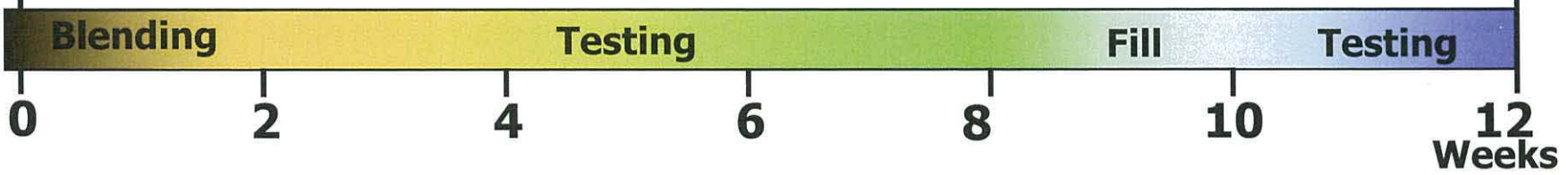


Start

- **Multivalent Vaccines**
- **Ranging from 3 in 1 to 6 in 1 Vaccines ± Wormer and/or Vitamin B12 and/or Selenium**



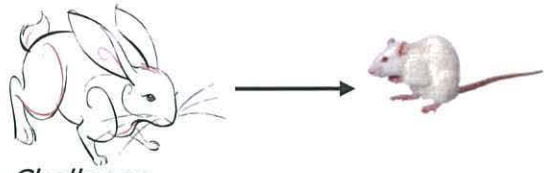
Release for Sale



- *Efficacy*

- Demonstrate using a model system that the product actually raises a response in animals using:

- *Serological Response*



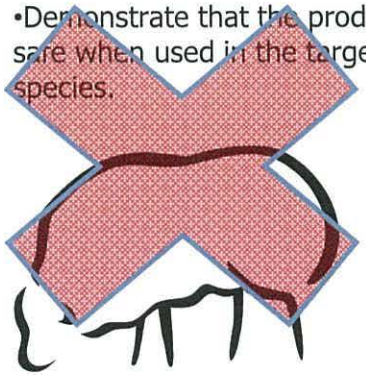
- *Challenge*

Pass

Fail

- *Target Safety*

- Demonstrate that the product is safe when used in the target species.



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Goal of the work

Current control tests are based largely on lab animal use:
mice, guinea pigs and rabbits

Goal to achieve within the next 10 years:
80% control tests based on ***in-vitro*** **tests**

20% in-vivo tests will be still required: for reagent calibration, in case of significative change of manufacturing process and for testing new vaccines

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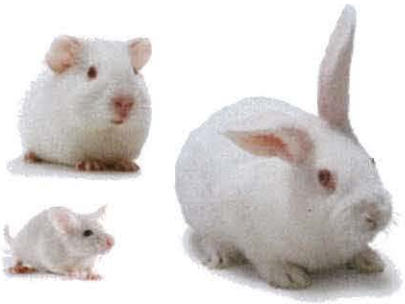
Vaccine complexity – a challenge



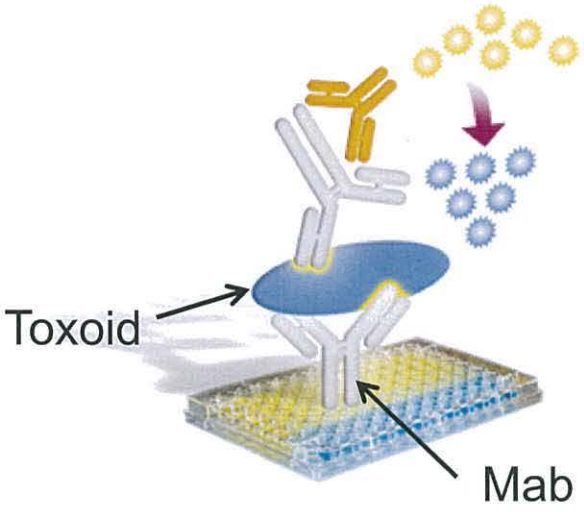
- 14 different vaccines
- 10 different antigens, mainly toxoids
- Vaccines contain 3 to 7 different antigens
- Using oil and aluminium adjuvants
- Some supplemented with moxidectin, selenium and/or Vit B12

Lead-time and capacity benefit only realised when you can test all antigens with new faster technology

Vaccine specificity – a challenge

Monoclonal antibodies must be specific to the toxoid we want to capture and quantify

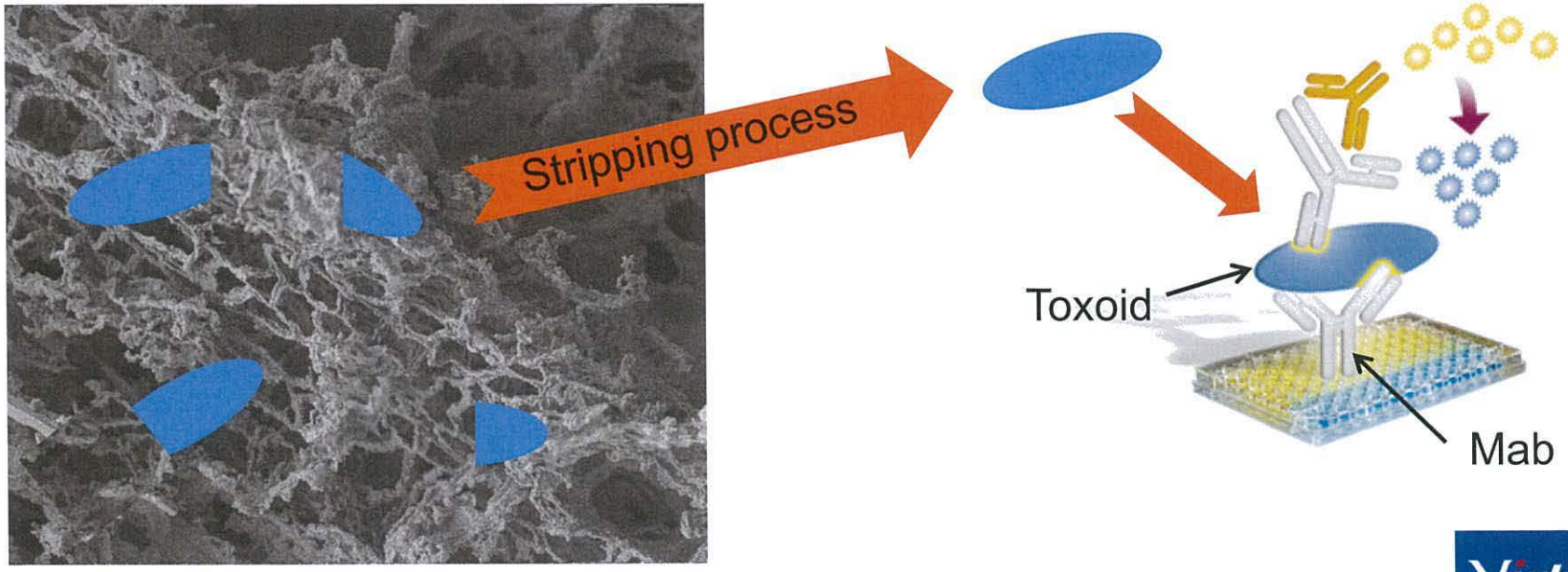
E.g. *Clostridium perfringens* D and *Corynebacterium pseudotuberculosis* both produce a phospolipase (toxoid) and are in the same vaccine. However the monoclonal is not specific ... *Grrrrrr*



Vaccine adjuvants – a challenge

If the toxoid must be stripped off the adjuvant to quantify it, this introduces more challenges

- Must you strip the toxoid, if so, how much?
- How reproducible is the stripping process?
- Does stripping affect the toxoid structure?



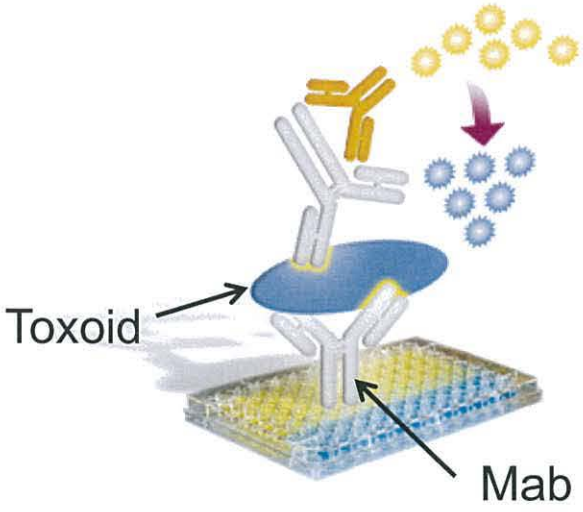
Vaccine assay validation – a challenge

Every new assay must be **validated**

- Specificity
- Linearity
- Limits of quantification
- Reproducibility (intra– and interassay)
- Accuracy
- Robustness

And supported by

- Controls and standards
- SOP
- Training of QC staff
- On-going support



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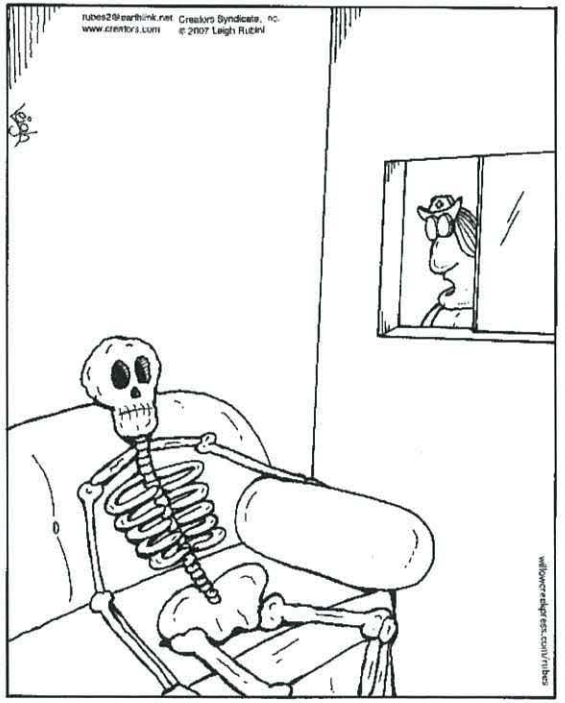
Parallel testing – a challenge

Every new assay will be **tested in parallel** with the existing animal test

However some vaccines we only make 1-2x/yr so testing 20 batches in parallel could take...

30 years !!

... Partially resolved by making extra lab batches



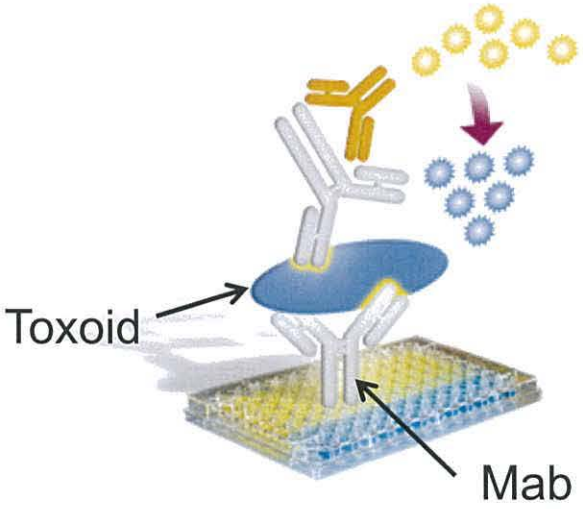
"The doctor will be with you in just five more minutes."

Vaccine assay Registration – a challenge

Every assay, for each of 10 antigens, will need to be registered against each product they are used in.

- Registration cost
- Registration risk

Australian Government
Australian Pesticides and Veterinary Medicines Authority



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