Elimination of target safety testing and the development of *In-vitro* assays for testing of livestock vaccines.

by Dr G McKay and Dr R P Dempster

Virbac

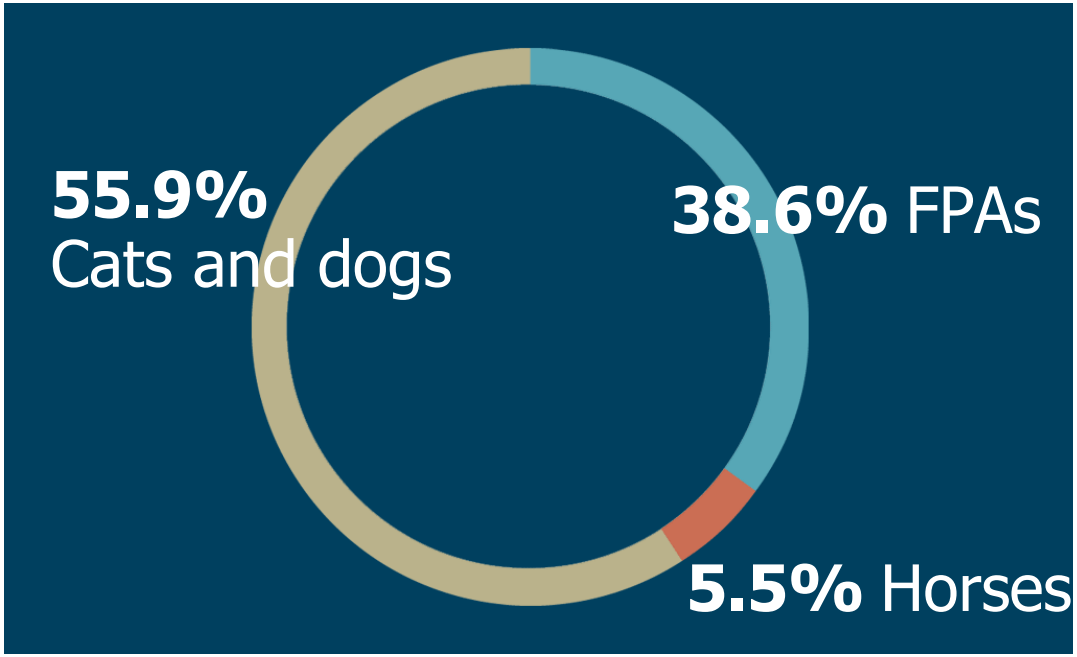
Introduction

Presentation Overview

- Introduction
- Regulatory background to vaccine testing requirements
- Overview of manufacturing process and associated testing.
- Current *in-vivo* Potency models used by Virbac Australia Pty Limited.
- Virbac's Goal and commitment to *in-vitro* test development.
- Planned strategy to replace *in vivo* testing.
- Our chance of getting the APVMA on side..
- Benefits of moving to Full *in-vivo* testing.
- Cost of the development program
- Questions. but please feel free to ask as we go.

Introduction

- Virbac is the largest international company dedicated exclusively to animal health
- Virbac produces a complete range of products and services for veterinarians and animal owners
- Virbac is active in all segments of animal health



Introduction

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

Product Categories, Markets and Regulatory Licensing

- Australia, New Zealand, Asia, South Africa and South America
  - Category 1 - Immunobiologicals and sterile products
  - Licensed by the APVMA under the **Manufacturers' Licensing Scheme** (Licence No: 1005) and audited at least every 24 months by an APVMA authorised auditor for compliance against the **Australian Code of Good Manufacturing Practice for Veterinary Chemical Products**.
- Europe:
  - Sterile products: Liquid dosage forms (Small Volume Parenterals) – aseptically prepared.
  - ***Certificate of GMP Compliance of a Manufacturer*** issued under the provisions of the Mutual Recognition Agreement between the EC and Australia (Certificate No: AU003V2006) and audited at least every 36 months by a TGA auditor for compliance against the **Australian Code of Good Manufacturing Practice for Medicinal Products.**
- USA
  - Tetanus Toxoid For Further Manufacture
  - **US Veterinary Biologics Establishment Licence** by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) under the Virus-Serum-Toxin Act (Licence No: 112-E); and audited at least every 24 months by the APHIS Veterinary Services (VS) Center for Veterinary Biologics (CVB) for conformity with the requirements in **Title 9 Code of Federal Regulations – Animal and Animal Products**.

Regulatory Guidelines for Animal Testing

- European Pharmacopoeia Guidelines
  - Requirements for testing and acceptance of vaccines are prescribed within the monographs for each vaccine type as exemplified below:

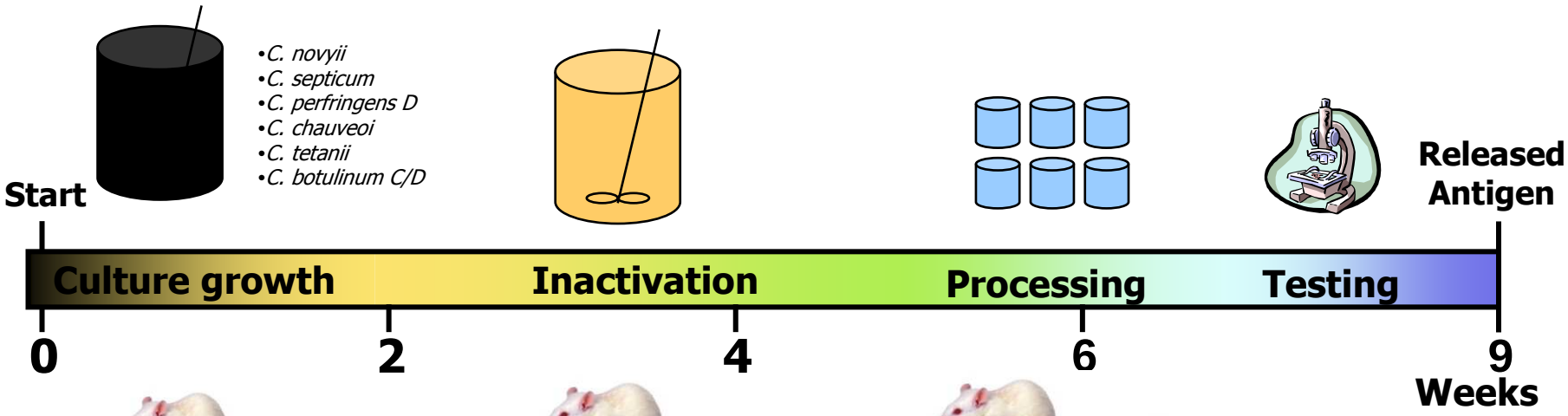
3-3. **Residual toxicity.** Inject 0.5 mL of the vaccine by the subcutaneous route into each of 5 mice, each weighing 17-22 g. Observe the mice at least daily for 7 days.

The vaccine complies with the test if no animal shows notable signs of disease or dies from causes attributable to the vaccine.

3-4. **Potency.** Use for the test not fewer than 10 healthy rabbits, 3-6 months old. Administer to each rabbit by the subcutaneous route a quantity of vaccine not greater than the minimum dose stated on the label as the 1st dose. After 21-28 days, administer to the same animals a quantity of the vaccine not greater than the minimum dose stated on the label as the 2nd dose. 10-14 days after the 2nd injection, bleed the rabbits and pool the sera.

The vaccine complies with the test if the potency of the pooled sera is not less than 2.5 IU/mL.

Antigen Production Process



Antigen Potency - Before Inactivation

L+ Test

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

Residual Toxicity

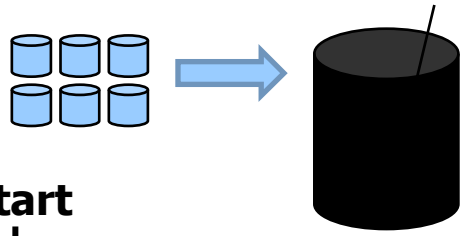
Demonstrates that the toxins are inactivated

Antigen Potency - Post Inactivation

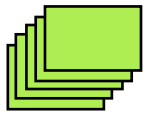
Total Combining Power Test

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

Vaccine Manufacturing Process

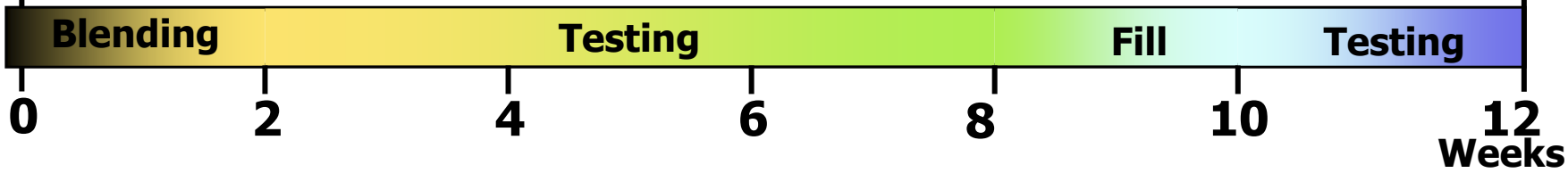


•Multivalent Vaccines
•Ranging from 3 in 1 to 6 in 1 Vaccines ± Wormer and/or Vitamin B12 and/or Selenium



Start

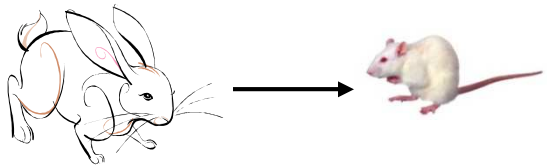
Release for Sale



•*Efficacy*

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

•*Serological Response*

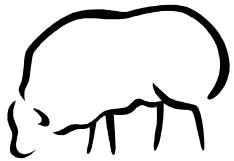


•*Challenge*

Pass *Fail*

•*Target Safety*

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

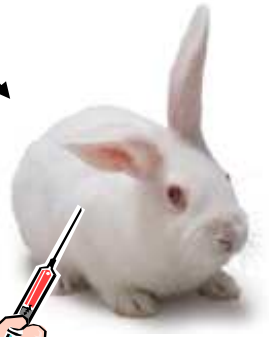


Virbac

Current *in-vivo* Potency Models used by Virbac

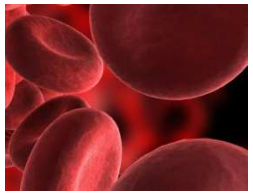
Serological Response Model

•Vaccinate T=0, T=4 weeks



•Bleed

•T=6 weeks



•Process Blood

•Combine

•KNOWN TOXIN

•& UNKNOWN ANTIBODY

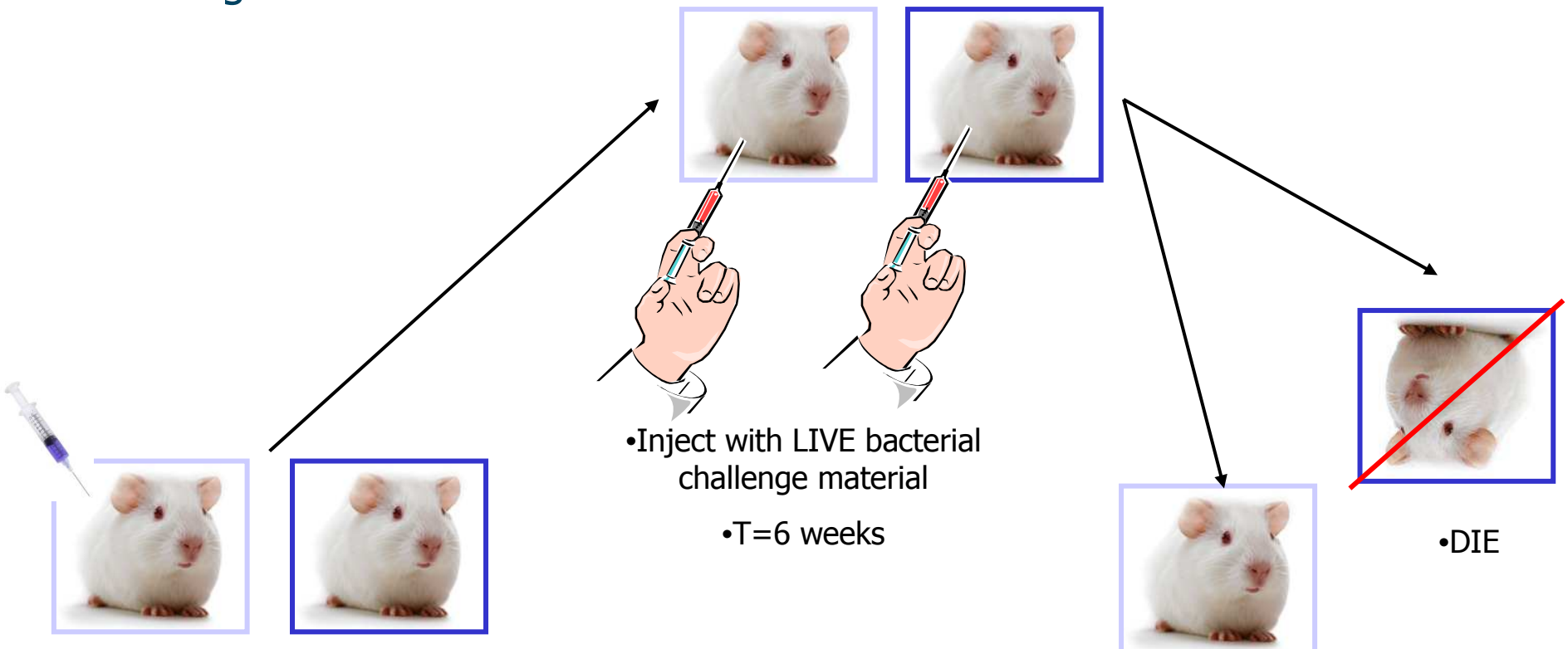
•Inject

•End Point

•50% dead / alive

Current *in-vivo* Potency Models used by Virbac

Challenge Model



•Vaccinates T=0, T=4 weeks

•Unvaccinated controls

•Inject with LIVE bacterial challenge material

•T=6 weeks

•SURVIVE

•DIE

In-vitro assay development for livestock vaccines

In-vivo

In-vitro

In-vitro assay development for livestock vaccines

Animal Research Review Panel's 2013 Animal Ethics Seminar

2nd October 2013

Goal of the work

Assurance of safety and potency for each batch of vaccine sold

Control tests are necessary

During manufacturing

On finished product

Based on lab animal use (rabbit, Guinea pigs, mice)

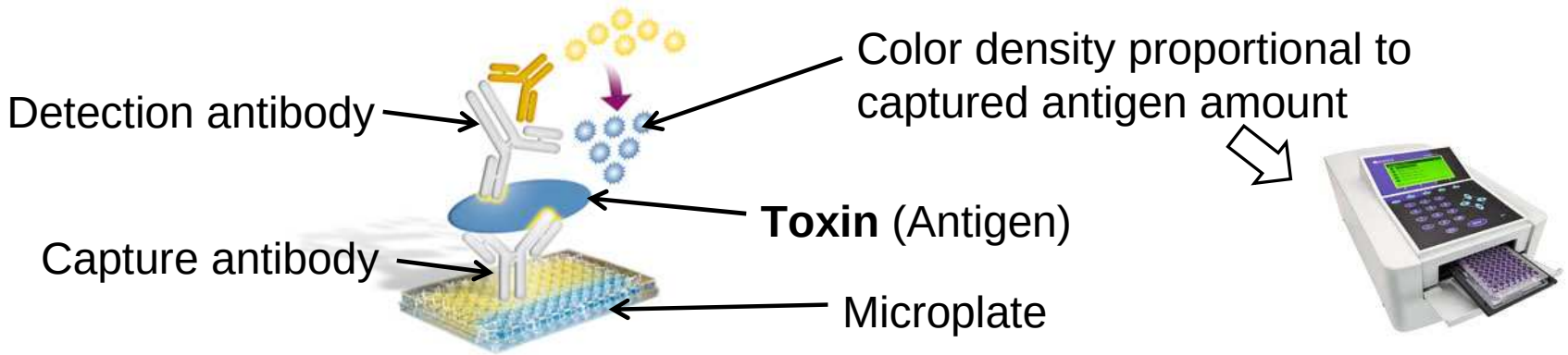
Goal for the next 5 years

Based on *in-vitro* assays

Planned strategy to replace *in-vivo* testing

~~L+ Test using mice~~ is used to quantify toxin

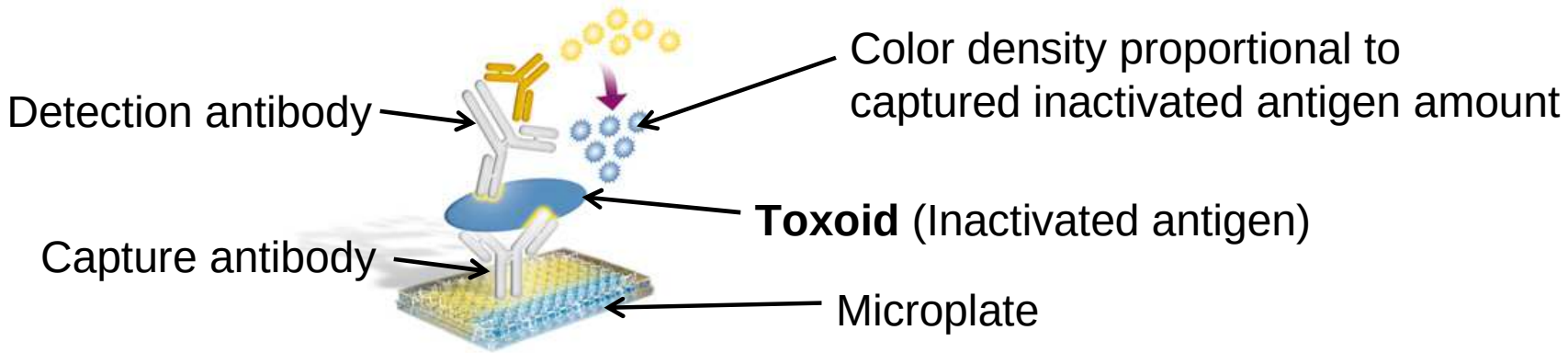
ELISA assay using antibodies specific to the antigen



Planned strategy to replace *in-vivo* testing

Total Combining Power Test using mice to quantify toxoid

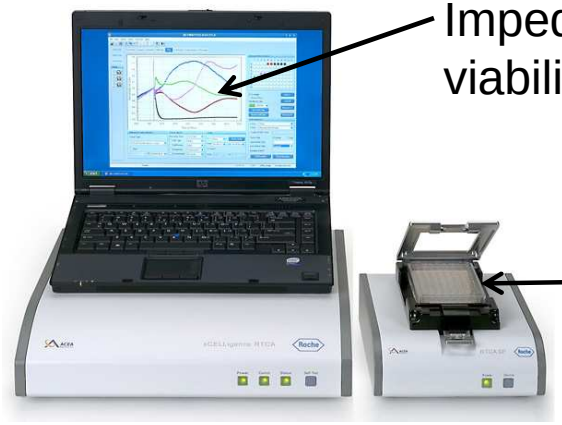
ELISA assay using antibodies specific to the inactivated antigen



Planned strategy to replace *in-vivo* testing

Residual toxicity test using mice or guinea pigs to confirm all toxin has been toxoided

Residual toxicity test using specific cell lines



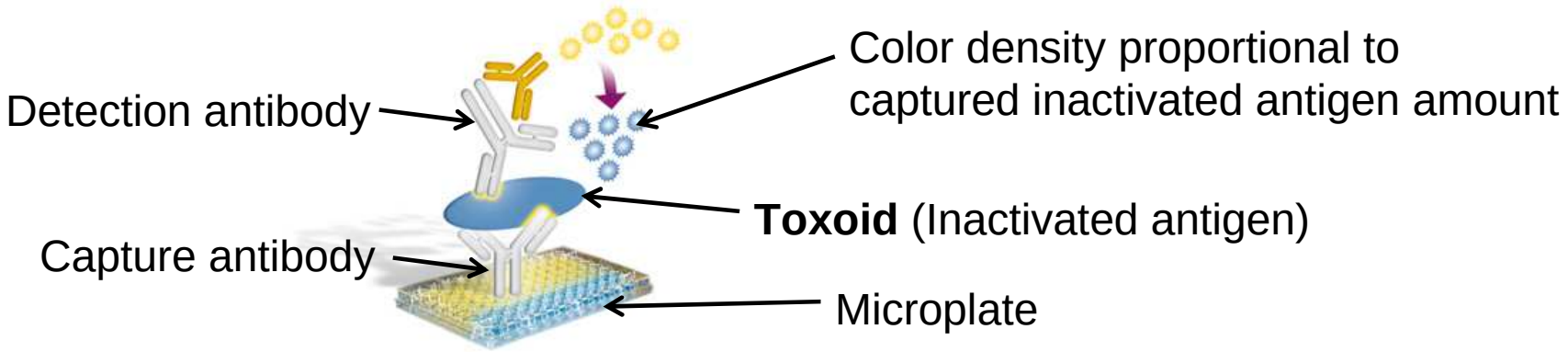
Impedance measured in wells proportional to cellular viability

Microplate with microelectrodes integrated into the bottom of each well containing the cells in culture

Planned strategy to replace *in-vivo* testing

SNT using Rabbits and mice to quantify antibody responses

ELISA assay using antibodies specific to the inactivated antigen

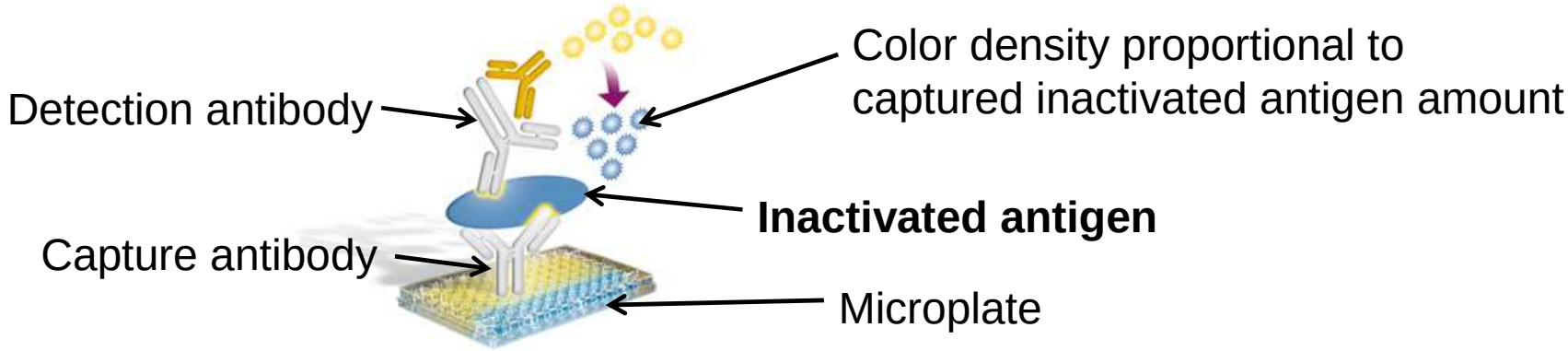


Verify an equivalent amount of inactivated antigen was integrated in vaccine by comparison with a reference vaccine previously demonstrated potent by *in-vivo* testing.

Planned strategy to replace *in-vivo* testing

Clostridium chauvoei **potency test is a challenge test using Guinea pigs**

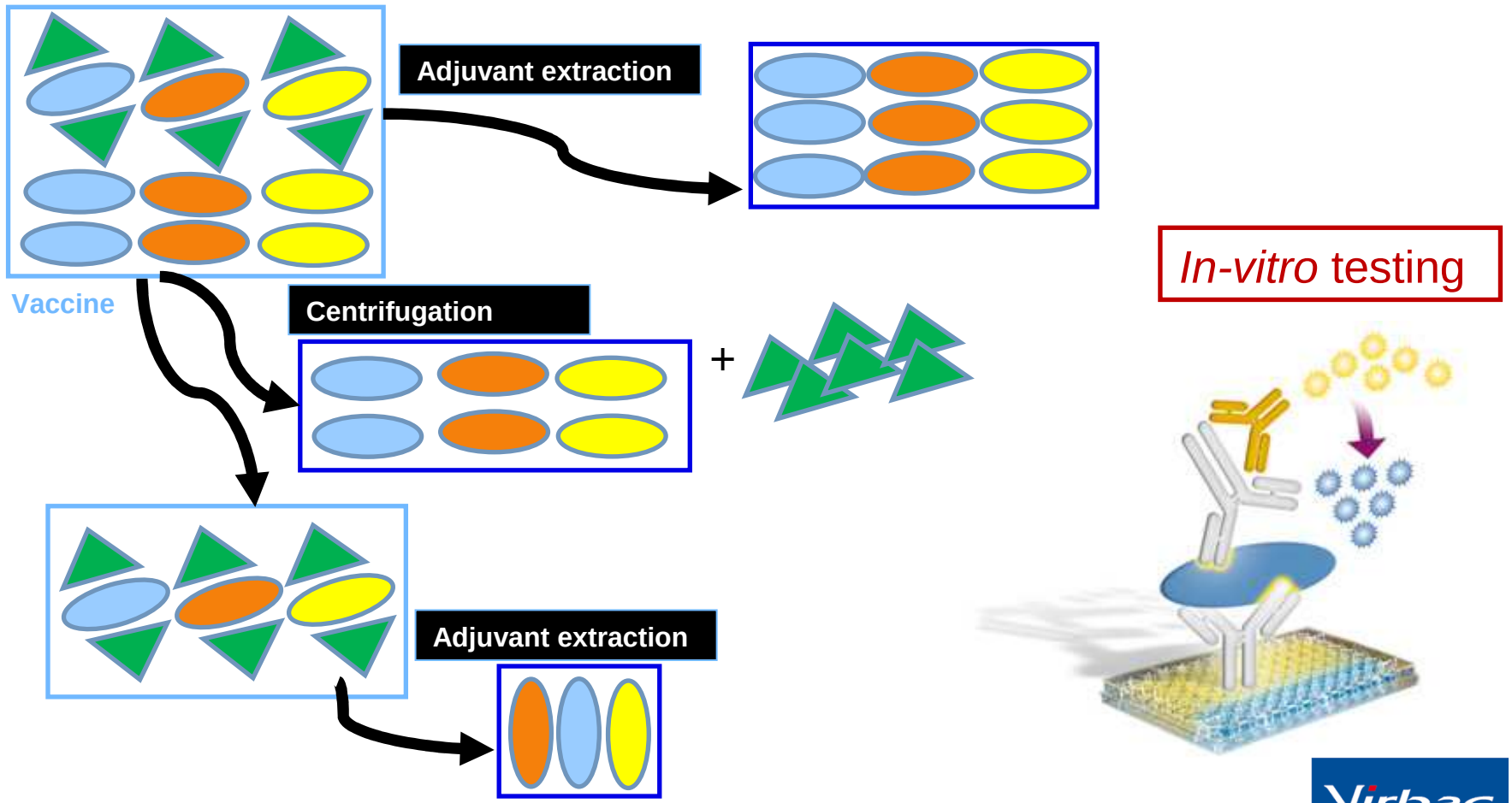
ELISA assay using antibodies specific to the protective antigen



Performed to verify an equivalent amount of inactivated antigen was integrated in vaccine by comparison with a reference vaccine previously demonstrated potent by in-vivo testing.

Planned strategy to replace *in-vivo* testing

How antigen-adjuvant interaction will be tested?



Our chances to get APVMA on side

- ✓**APVMA is already receptive**
- ✓**APVMA's knowledge about *in-vitro* testing built on Virbac's teaching**
- ✓**Scientific support of globally recognized organisations**
  - In USA: CVB USDA, NICEATM, CCVAM
  - In Europe: PEI, EPAA
- ✓**Highly qualified specific suppliers**
  - Specific antibodies:
    - Monash Antibody Technologies Facility (Melbourne, Australia)
    - In-Cell-Art (Nantes, France)
    - BioCytex (Marseille, France)
  - Cell lines: ODESIA NeoSciences (Sophia Antipolis, France)
- ✓**Highly qualified collaborators:**
  - Antigen purification: EMAI (Menangle, Australia)
  - Adjuvant Extraction methods: IDRI (Seattle, USA)

The benefits of *in-vitro* assays

Tests results obtained quicker

↓

Lead-time reduction of vaccine held in vessels before release for packing and sale = 8-week saving

↓ ↓ ↓

Vessels available for faster re-use

↓

✓ **Increase in plant capacity**

✓ **Virbac more responsive when competitor stocks out**

✓ **Reduction of backorder risk**

The benefits of *in-vitro* assays

Improve QC testing reliability

More accurate and reliable antigen test results (less error)

✓ **Less antigen wastage**

More accurate and reliable vaccine test results (less error)

✓ **Failed batches don't fail due to the test**

The benefits of *in-vitro* assays

- ✓ Virbac more responsive when competitor stocks out
- ✓ Failed batches don’t fail due to the test
- ✓ Reduction of backorder risk
- ✓ Increase in plant capacity
- ✓ Less antigen wastage
- ✓ $400,000 yearly cost saving
- ✓ Growth of Virbac's reputation
- ✓80% reduction of QC animal use

The Costs

- Virbac has bought out a PhD (Dr Nadine Hassaine) from France
- Programme expected to run for at least 5 years
- Total budget about $1.5m