

Considerations for Animal Use and Sustainability – A Regulatory Perspective

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Presentation Overview

- Overview of animal testing for product development and release testing.
 - Alternative test development related to 3Rs
- Animals and sustainability
 - Rabbit pyrogen test
 - Limulocyte activation test
 - Other examples:
 - Squalene used for adjuvant production
 - NHPs used for vaccine testing
- Role for regulators in the sustainability discussion

Important to remember!

- Research is essential!
 - Development of new technologies and new ways to produce vaccines improves our ability to make available state of the art vaccines
- Evolution is important!
 - We need to create and utilize pathways that allow for the introduction of new technologies while retaining safety and purity of vaccines
- The best efforts are collaborative efforts
 - Including academic, industry, and regulatory participation
- Science drives regulatory decision making

Regulations, Quality Control, and Sustainability

- Quality control testing must:
 - Ensure the safety, purity, and potency of the product
 - Code of Federal Regulations (for US)
 - Demonstrate a consistent manufacturing process
 - Adherence to cGMPs
 - Rely on the best technology available
 - Assays need to be fit for purpose
 - Be flexible to allow evolution of testing programs
 - Often difficult to incorporate new technology
 - Barriers include inconsistency in regulatory expectations
 - Make room for the principles of 3Rs
 - **Start to consider sustainability issues?**
 - **Material use and carbon footprint**
 - **Animal use**

CBER engagement in 3Rs - Examples

- **Reduction of animal testing**
 - Removal of the requirement for the General Safety Test (GST)
- **Refinement of animal testing**
 - Mumps vaccine
 - Monkey neurovirulence vs. rat neurovirulence
- **Replacement (substitutions) of animal testing**
 - Adventitious agents testing
 - Multiple animal assays vs. high throughput sequencing (HTS)
 - DTaP vaccine
 - Lethal histamine sensitization assay in mice vs. *in vitro* CHO cell-based assay
 - Inactivated poliovirus vaccine
 - Rat immunogenicity vs. antigen ELISA
 - HepB virus, HPV and other vaccines
 - Immunogenicity in mice vs. ELISA
 - Inactivated rabies virus vaccine
 - Lethal challenge in mice vs. antigen ELISA

Alternate test development and sustainability - 1

- Many ongoing efforts to reduce animal use in testing
- Sustainability has not been a critical part of the discussion
- Two critical issues related to animal use and sustainability to consider
 - Use of animal raised in breeding facilities
 - Use of animals when alternative assays are available
 - Energy and resources used to maintain animal facilities
 - Use of wild caught animals
 - Use of animals when alternative assays are available
 - Endangerment of animal species
 - Endangerment of habitat and impact on other animal species

Alternate test development and sustainability - 2

- Benefits of alternative test development to sustainability for animals provided from controlled breeding facilities
 - Rabies NIH Potency test vs ELISA method under development
 - 300+ animal used per test (in US)
 - Test can take up to six weeks to complete
 - Multiple retests based on validity criteria not being met
 - Rabbit pyrogen test vs MAT assay
 - Hundreds of thousands of rabbits used annually

Environmental impact of animal use - 1

Bacterial endotoxin testing - LAL test

- Based on blood collected from wild caught horseshoe crabs
 - 4 species of crabs: 3 in Asia, 1 in North America
- North American species is listed as “vulnerable” with respect to population numbers
 - No restrictions on collection and use
- More than 500,000 crabs are caught and bled annually
- Impacts horseshoe crab population as well as shore birds that have evolved a migration time and route to coincide with egg laying by horseshoe crabs to sustain the bird migratory bird population

PLOS Biology | <https://doi.org/10.1371/journal.pbio.2006607> October 12, 2018



Alternative to LAL testing

Recombinant Factor C:

- Bacterial endotoxin test based on recombinant factor C
 - Factor C is the first protein involved in the cascade of events that leads to LAL read out from horseshoe crab blood.
 - Endotoxin testing using recombinant factor C has been shown to be as sensitive as LAL testing.
 - Introduced over ten years ago.
 - How do we encourage the change to recombinant factor C use?

Environmental impact of animal use - 2

Non-human primates for pre-clinical testing and product development

- Used for safety and biodistribution studies
- Used for vaccine challenge studies
- Historically, animals were wild caught and imported.
 - This practice impacted the natural populations and habitats for many animals, destroyed family units, impacted population numbers
- Current regulations for primates used for testing include a requirement that imported animals have paperwork to ensure provenance and that they have been bred for laboratory use, however:
 - During the COVID epidemic the need for NHPs was so great that some Asian suppliers implemented the practice of using wild caught NHPs

Alternatives to use of NHPs

- In vitro methods
- Other relevant small animal models
- Organ on a chip technology
 - Shown to be equivalent or better than current animal models
 - From a time, sustainability, and financial point of view

Environmental impact of animal use - 3

Squalene used for adjuvant production

- Sourced from shark liver oil
- Severe over-fishing of shark population in general
 - In addition, the synthesis of other by products such as squalene lead to further depletion

Alternatives to shark liver oil for squalene synthesis

- *Kilo-Scale GMP Synthesis of Renewable Semisynthetic Vaccine-Grade Squalene*
- Development of yeast that produce the sesquiterpene *trans-β*-farnesene which is now produced commercially by fermentation of sustainably grown sugar cane.
- *Trans-β*-farnesene is used for the commercial semi-synthesis of squalene used as an emollient
- Used as the precursor for a series of squalene analogues, some of which display vaccine adjuvant properties equivalent or superior to squalene when appropriately formulated in o/w emulsions.

► Ph. Eur. exercise to phase out the rabbit pyrogen test



Towards animal free pyrogen tests in the Ph. Eur: latest progress



11th PharmaLab Congress – Endotoxin and Pyrogen Testing
Düsseldorf, 21-22 November 2023

Gwenael Cirefice, EDQM
Biologicals 84 (2023) 101702

Assays for pyrogens / endotoxins in the Ph. Eur.

1971



Pyrogens (2.6.8)
("Rabbit Pyrogen Test")



Pyrogen detection

1987



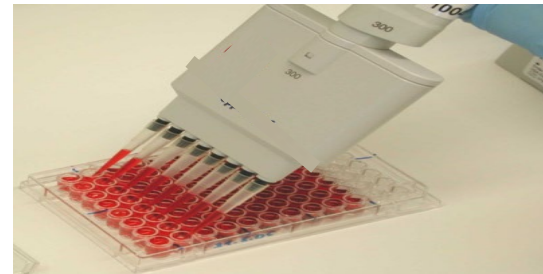
LAL is a lyophilised amoebocyte lysate obtained from the horseshoe crab (*L. polyphemus* or *T. tridentatus*)

BET (2.6.14) &
Guidelines for using the
BET (5.1.10)



Endotoxin detection

2010



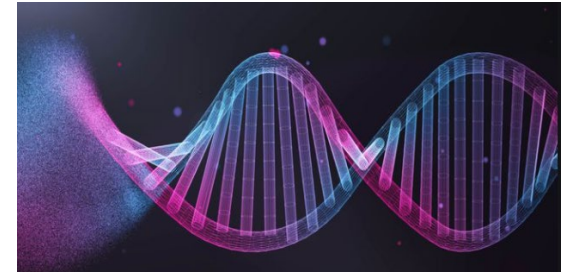
Monocyte-activation test
(2.6.30)

▶ *MAT for vaccines containing inherently pyrogenic components (2.6.40) [NEW]*



Pyrogen detection

2020





BET using recombinant
Factor C (2.6.32)



Endotoxin detection

Timelines



WHAT	WHO	WHEN	
		Publication in PhPa <input checked="" type="checkbox"/>	Envisaged implementation date
 Elaboration of new chapter on Pyrogenicity (5.1.13) (and revision of chapter 5.1.10)	BET WP	●	●
REVISION			
<i>Chapter 2.6.30</i>	BET WP	●	●
<i>Gen. monograph 2034</i>	BET WP	●	●
<i>Gen. monograph 0520</i>	G12 with BET WP support	●	●
All other Ph. Eur. texts	GoE/WP with BET WP support	●	●
 Pyrogens (2.6.8)			●

