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Development of MAT for Plasma-derived Medicinal Products in South Korea

25 Sep 2024

Ministry of Food and Drug Safety

Background

Ministry of Food and Drug Safety

Global Attribution for Alternative Test Methods

Globally rising awareness of animal ethics has highlighted the importance of alternative test methods.

Direction for Alternative Animal Test in Korea

- ✓ **Continued efforts to develop standardized the alternative test methods for drug development and quality control**
- ✓ **Collaborative activities with OECD, ISO, etc. to develop and harmonize guidelines on the alternative test methods**

Background

Ministry of Food and Drug Safety

PDMPs under national lot release in 2023, Korea

Human Tetanus Immunoglobulin Potency Test

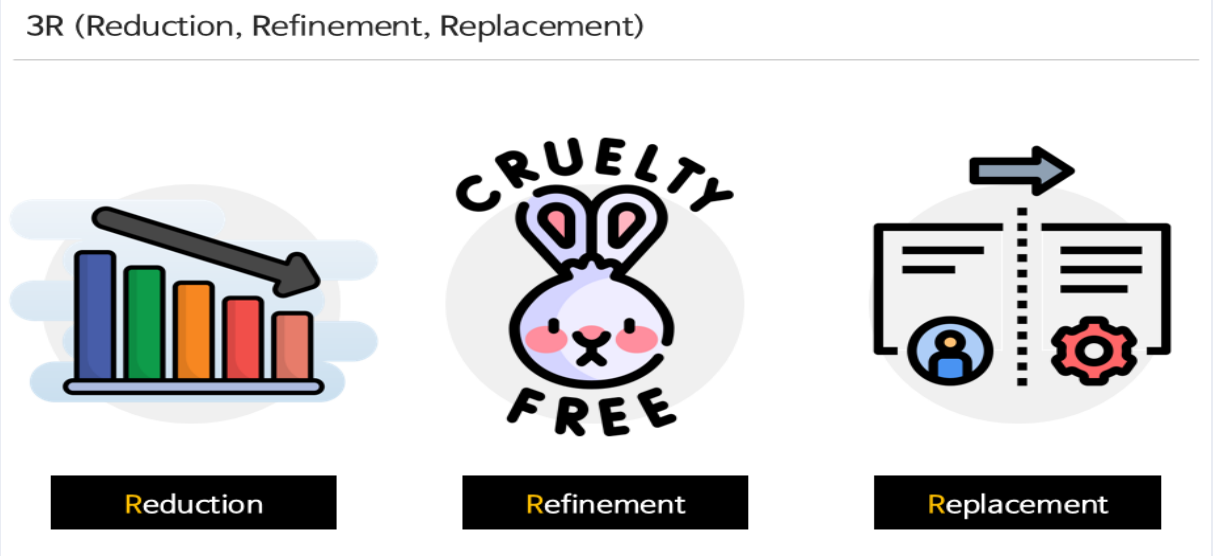
Name of preparation (according to the MRBP)	Number of products
Human Serum Albumin	4
Human Normal Immunoglobulin in Glycine	2
Human Normal Immunoglobulin in Maltose	2
Human Normal Immunoglobulin	1
Human Hepatitis B Immunoglobulin for Intravenous Administration	2
Human Hepatitis B Immunoglobulin	1
Human Tetanus Immunoglobulin	2
Human Varicella Immunoglobulin	1
Freeze-dried Human Normal Immunoglobulin with Histamine	1
Freeze-dried Concentrated Human Blood Coagulation Factor VIII (Dry Heat Treated)	2
Factor VIII:C Monoclonal Antibody-purified, Freeze-dried Human Blood Coagulation Factor VIII:C	2
Factor VIII Inhibitor Bypassing Activity Complex	1
Freeze-dried Human Blood Coagulation Factor IX Complex	1
Freeze-dried Concentrated Human Anti-thrombin III	2
Freeze-dried Human Fibrinogen	1
Fibrin Sealant Kit	8

Animal Testing for Lot Release of PDMPs in Korea

- Rabbits are used for pyrogen test

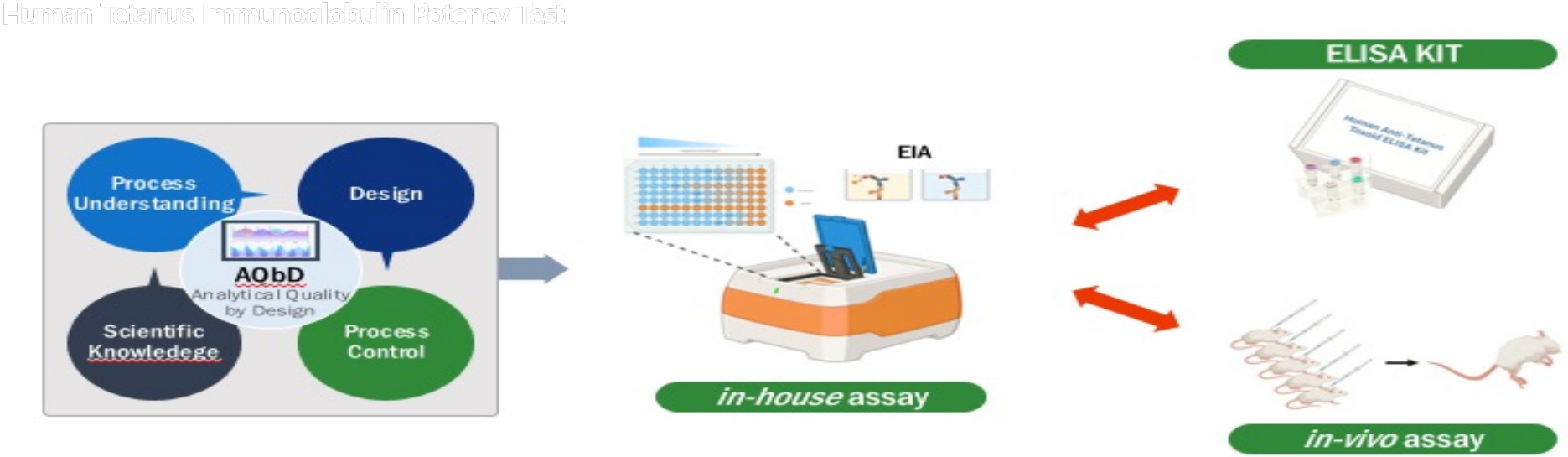
  : 28 out of 33 test items (80%) for PDMPs in South Korea

- Mice are used to test the potency of human anti-tetanus immunoglobulins
- Mice and rabbits are used to test the potency of dried Mamushi antivenom.



R&D History : Alternatives to animal testing for QC of Biologics

Potency Test for Human Tetanus Immunoglobulin



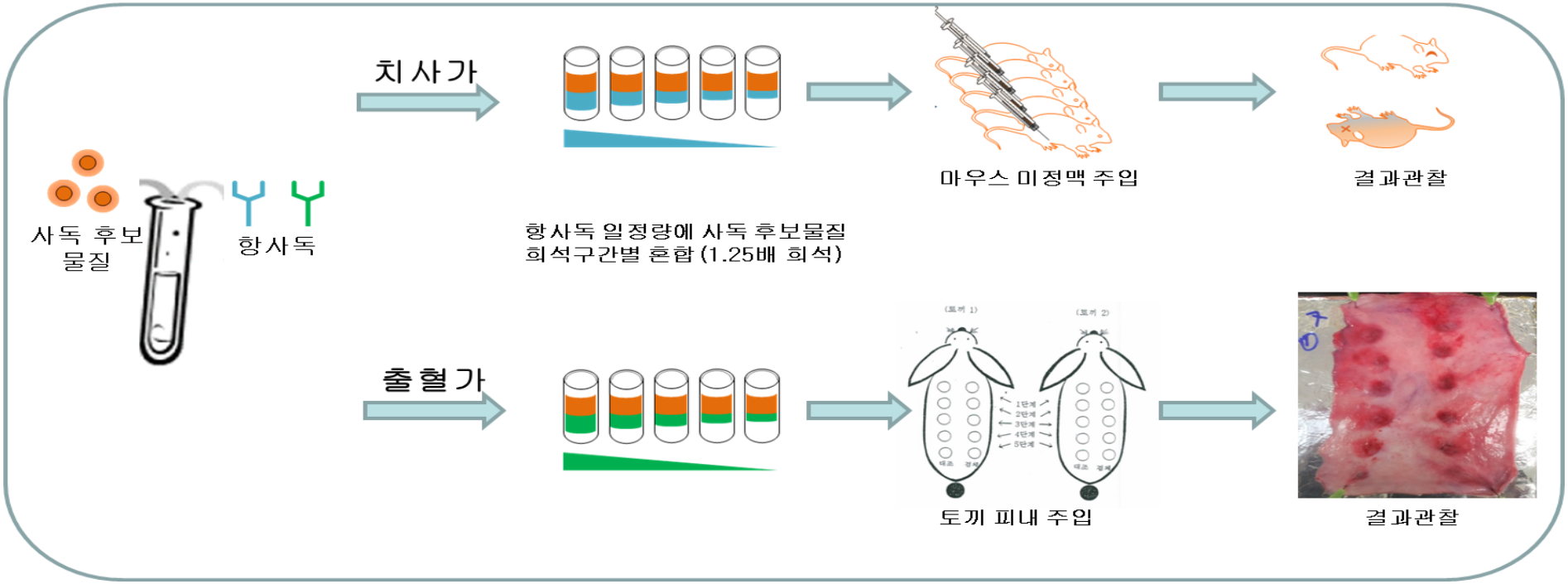
- Study on the *in vitro* potency testing of human anti-tetanus immunoglobulin (2012)
- Ongoing research to establish and validate alternative test methods for the potency testing of human anti-tetanus immunoglobulin (2023 -)

  -> Study to validate some *in vitro* potency assay kits (2024 -)

R&D History : Alternatives to animal testing for QC of Biologics

Potency Test for Antivenom

Human Tetanus Immunoglobulin Potency Test

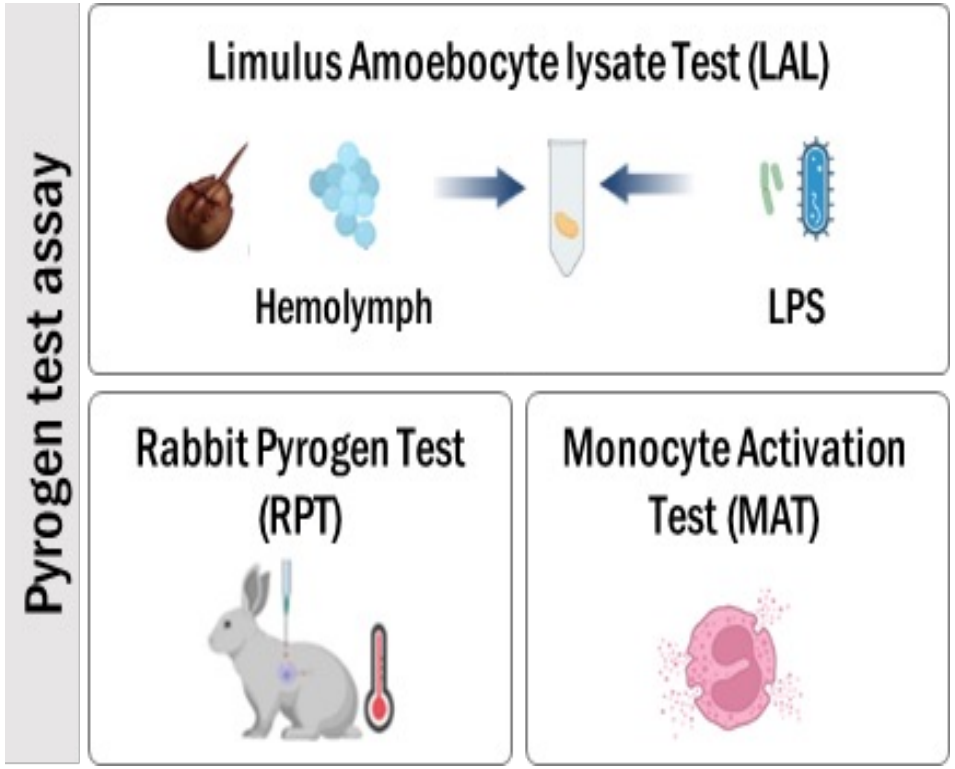


- Established the national standard of venoms/antivenoms for potency test (-2018)
- Research project to develop an alternative test methods (EIA) (2024 -)

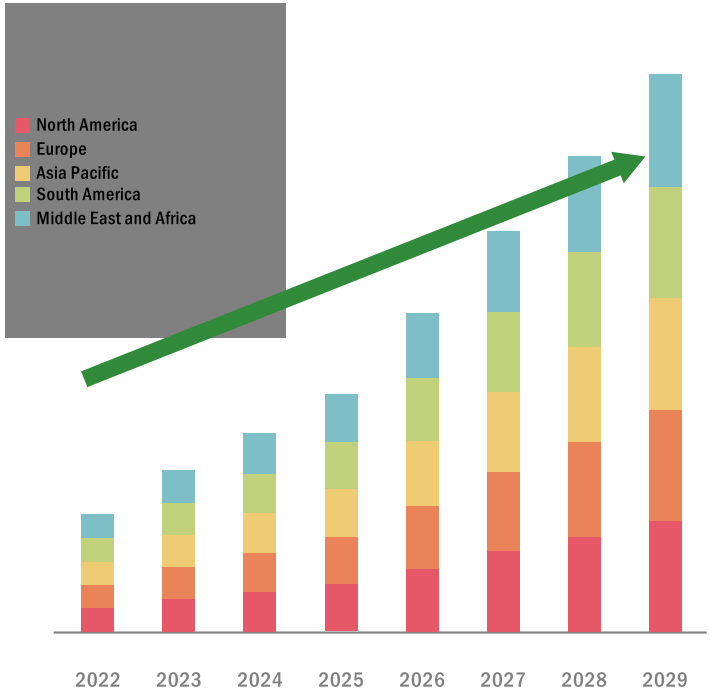
R&D History : Alternatives to animal testing for QC of Biologics

Pyrogen Test

Human Tetanus Immunoglobulin Potency Test



Global Pyrogen Testing Market is Expected to Account for USD 2,784.75 Million by 2029



Source: Date Bridge Market Research Market Analysis study 2022

R&D History : Alternatives to animal testing for QC of Biologics

Monocyte Activation Test (MAT)

Biologicals 71 (2021) 20–30

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Research paper

Development of a rabbit monocyte activation test as an alternative to the rabbit pyrogen test and its application in the analysis of plasma-derived products

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ABSTRACT

The rabbit pyrogen test (RPT) is a safety test conducted as a part of mandatory requirements of regulatory agencies. RPT is currently performed for routine quality control (QC) by manufacturers and for national lot release of biological products, such as plasma-derived products. However, RPT involves the use of many rabbits, counter to the international efforts to minimize the use of animals in research. Furthermore, pyrogen amount cannot be discerned from the test results and the results may be considerably affected by various factors. Therefore, a need exists for substituting RPT with *in vitro* assays. As a viable alternative to RPT, we here established a rabbit monocyte activation test (RMAT) based on the human MAT in the European Pharmacopoeia. RMAT uses rabbit peripheral blood mononuclear cells as the source of monocytes instead of live animals. The test detected endotoxin, lipoteichoic acid, peptidoglycan, and zymosan with high sensitivity, showing high correlation with the *in vivo* RPT results. The results of RMAT and RPT testing of non-pyrogenic plasma-derived products were also consistent. Furthermore, RMAT showed satisfactory recovery rates in an interference test with product samples and spiked-in pyrogens. We conclude that RMAT could replace the existing RPT for routine QC.

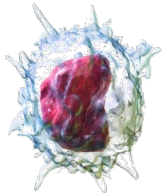
Pyrogens

1. Endotoxins
(LPS from gram negative bacteria)

2. Non-endotoxin pyrogens
(gram-positive bacteria, yeaset, mold, virus, etc)

→

Monocyte



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Cytokines

IL-1β,
IL-6,
TNF-α,
IFN-γ

→

Detection of IL-6 with ELISA

- **It is highly sensitive and accurate.**
- **It is not subject to variability between rabbit species.**
- **It does not raise ethical concerns.**
- **It is capable of detecting all types of pyrogens.**

- **The MFDS did fundamental research to develop an in vitro MAT using rabbit monocytes (2016–2018).**
  -> The rabbit monocytes still have some limitations. More research efforts and safety data are required.
- **Our new study aims to establish an MAT for national lot release testing (2023-).**

Capability evaluation and performance

(Revision) Specifications and Test Methods for Biologicals

Delete Abnormal Toxicity Test (2022.8.)

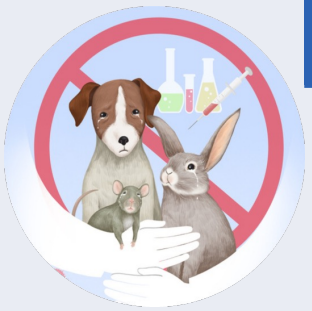
(Revision) Korean Pharmacopoeia

Add Recombinant factor C (rFC) Assay (2023.9.)

(Revision) Specifications and Test Methods for Biologicals

Delete Antihemorrhagic Titer, one of Potency Tests from Freeze-dried Agkistrodon Antivenom (2024.9.)

Allow Single Dilution Assay for Diphtheria and Tetanus Potency Test (2024.9.)
- limited to validity proven



(Revision) Korean Pharmacopoeia

Add Monocyte Activation Test, alternatives to Rabbit Pyrogen Test (~2025.)

Add LC-MS Method, alternatives to Histamine Test, and Change Cat into Guinea pig in Histamine method (~2025.)

Thank You

Ministry of Food and
Drug Safety