IMMUNOLOGY UNIT

DEPARTMENT OF INFECTIOUS DISEASES

ISTITUTO SUPERIORE DI SANITÀ

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ISTITUTO SUPERIORE DI SANITÀ

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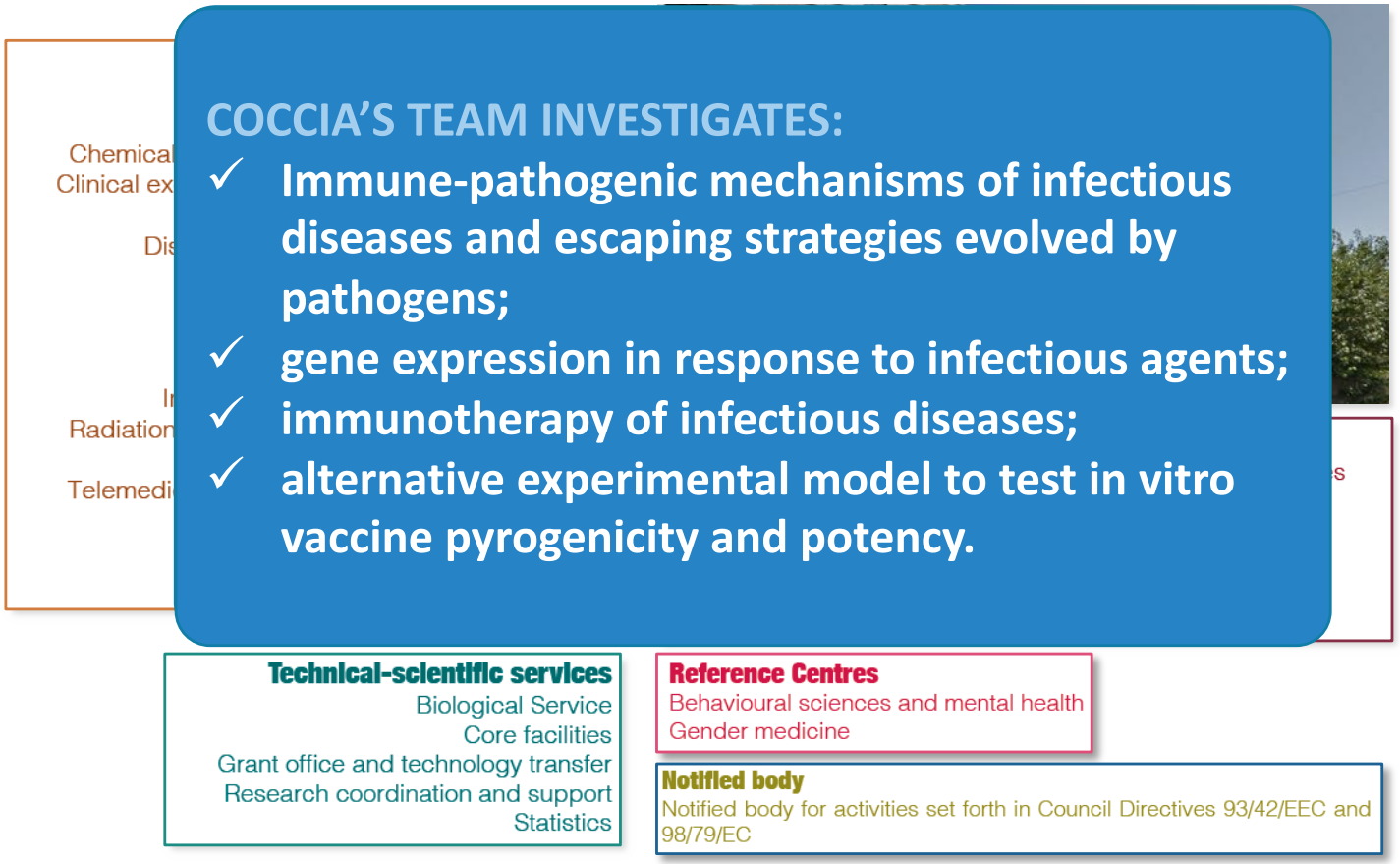
MISSION: Promotion and protection of national and international public health through research, surveillance, regulation, control, prevention, communication, counseling and training activities.



ALTEX 31, 4/14

A Human Dendritic Cell-Based *In Vitro* Model to Assess *Mycobacterium Tuberculosis* SO2 Vaccine Immunogenicity

Marilena P. Etna [1], Elena Giacomini [1], Martina Severa [1], Manuela Pardini [1], Nacho Aguilo [2,3], Carlos Martin [2,3,4] and Eliana M. Coccia [1]

SCIENTIFIC REPORTS

OPEN

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Impact of *Mycobacterium tuberculosis* RD1-locus on human primary dendritic cell immune functions

Marilena P. Etna[1], Elena Giacomini[1], Manuela Pardini[1], Martina Severa[1], Daria Bottai[2,3], Melania Cruciani[1], Fabiana Rizzo[1], Raffaele Calogero[4], Roland Brosch[3] & Eliana M. Coccia[1]

frontiers in Immunology

ORIGINAL RESEARCH
published: 12 November 2019
doi: 10.3389/fimmu.2019.02622

Differential Responses of Human Dendritic Cells to Live or Inactivated *Staphylococcus aureus*: Impact on Cytokine Production and T Helper Expansion

OPEN ACCESS

Melania Cruciani [1], Silvia Sandini [1†], Marilena P. Etna [1†], Elena Giacomini [1], Romina Camilli [1], Martina Severa [1], Fabiana Rizzo [1], Fabio Bagnoli [2], John Hiscott [3] and Eliana M. Coccia [1]*

frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: 21 July 2017
doi: 10.3389/fcimb.2017.00330

Staphylococcus aureus Esx Factors Control Human Dendritic Cell Functions Conditioning Th1/Th17 Response

Melania Cruciani [1,2†], Marilena P. Etna [2†], Romina Camilli [2], Elena Giacomini [2], Zulema A. Percario [1], Martina Severa [2], Silvia Sandini [2], Fabiana Rizzo [2], Valentina Brandi [1], Giuliana Balsamo [3], Fabio Polticelli [1,4], Elisabetta Affabris [1], Annalisa Pantosti [2], Fabio Bagnoli [3] and Eliana M. Coccia [2]*

vac2vac

- http://www.imi.europa.eu/
- http://www.vac2vac.eu/

VAC2VAC - Vaccine batch to vaccine batch comparison by consistency testing

OBJECTIVES AND AMBITION

- ✓ Develop, optimise & evaluate non-animal methods that cover key-parameters for demonstrating vaccine batch consistency, safety and efficacy.
- ✓ (Pre-)validate methods and define with regulators guidance for regulatory approval and routine use.

FUNDING

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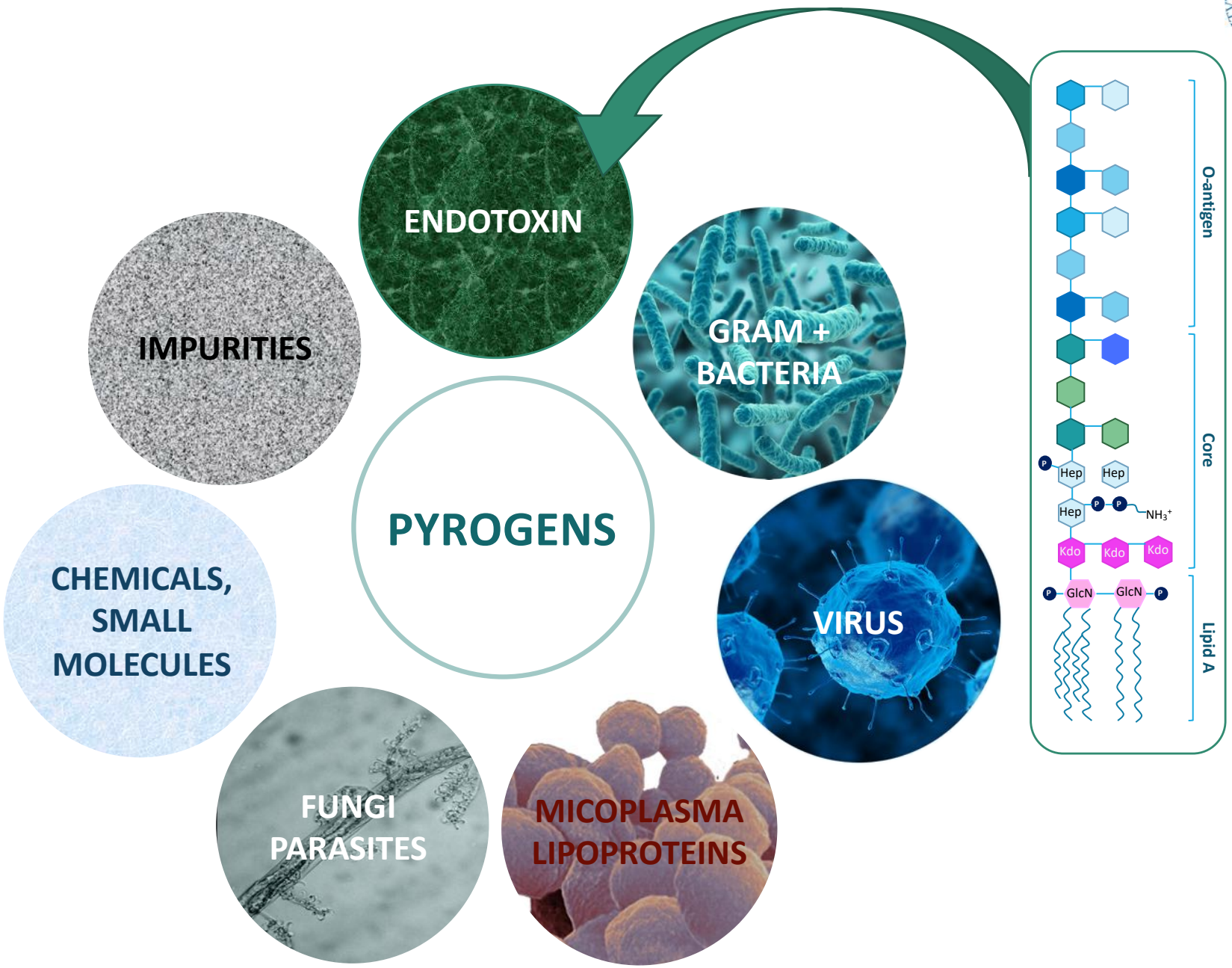
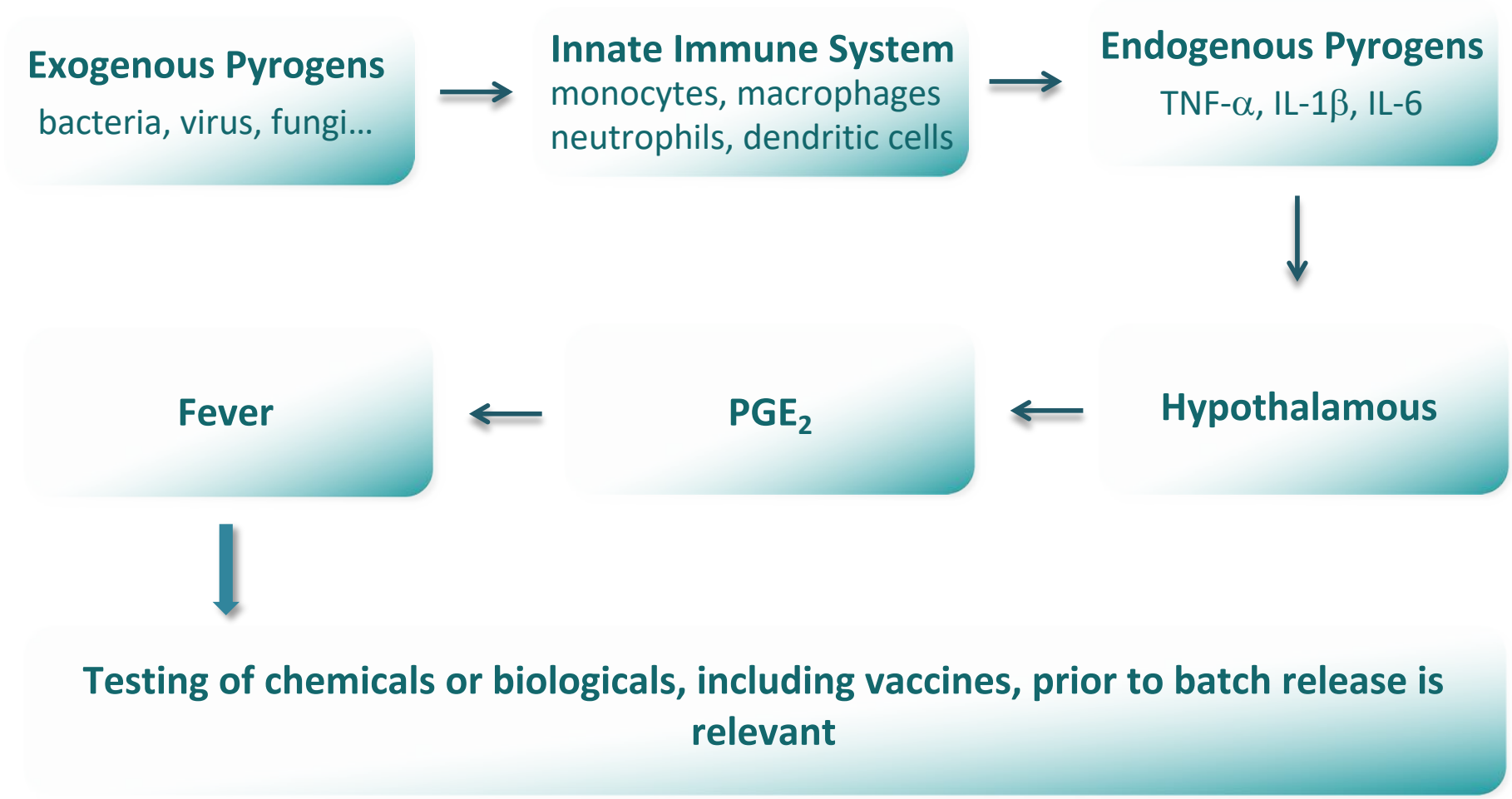
This presentation reflects only the author's views and the European Union is not liable for any use that may be made of the information contained therein.

Monocyte activation test: an *in vitro* method to evaluate the pyrogenic content of human vaccine

Eliana M. Coccia and Marilena P. Etna
Dept. of Infectious Diseases
Immunology Unit


ENDOTOXIN
IMPURITIES
GRAM +
BACTERIA
PYROGENS
CHEMICALS,
SMALL
MOLECULES
VIRUS
FUNGI
PARASITES
MICOPLASMA
LIPOPROTEINS
O-antigen
Core
Lipid A
Hep
Hep
Hep
Kdo
Kdo
Kdo
GlcN
GlcN
NH3+

PYROGENS: WHAT THEY TRIGGER?



HOW WE CAN TEST THE PYROGEN CONTENT OF A PRODUCT?

- ✓ Rabbit Pyrogen test [RPT]
- ✓ Bacterial Endotoxin test [BET]
- ✓ Recombinant Factor C test [rFC]
- ✓ Monocytes Activation Test [MAT]

HOW TO CHOOSE AMONG THE DIFFERENT METHODS?

PYROGEN/ENDOTOXIN TESTS (I)

RPT- Rabbit pyrogen test

(Qualitative measurement of endotoxin and non-endotoxin pyrogens)

"The test consists of measuring the rise in body temperature evoked in rabbits by the intravenous injection of a sterile solution of the substance to be examined" (Chapter 2.6.8 Ph. Eur.).

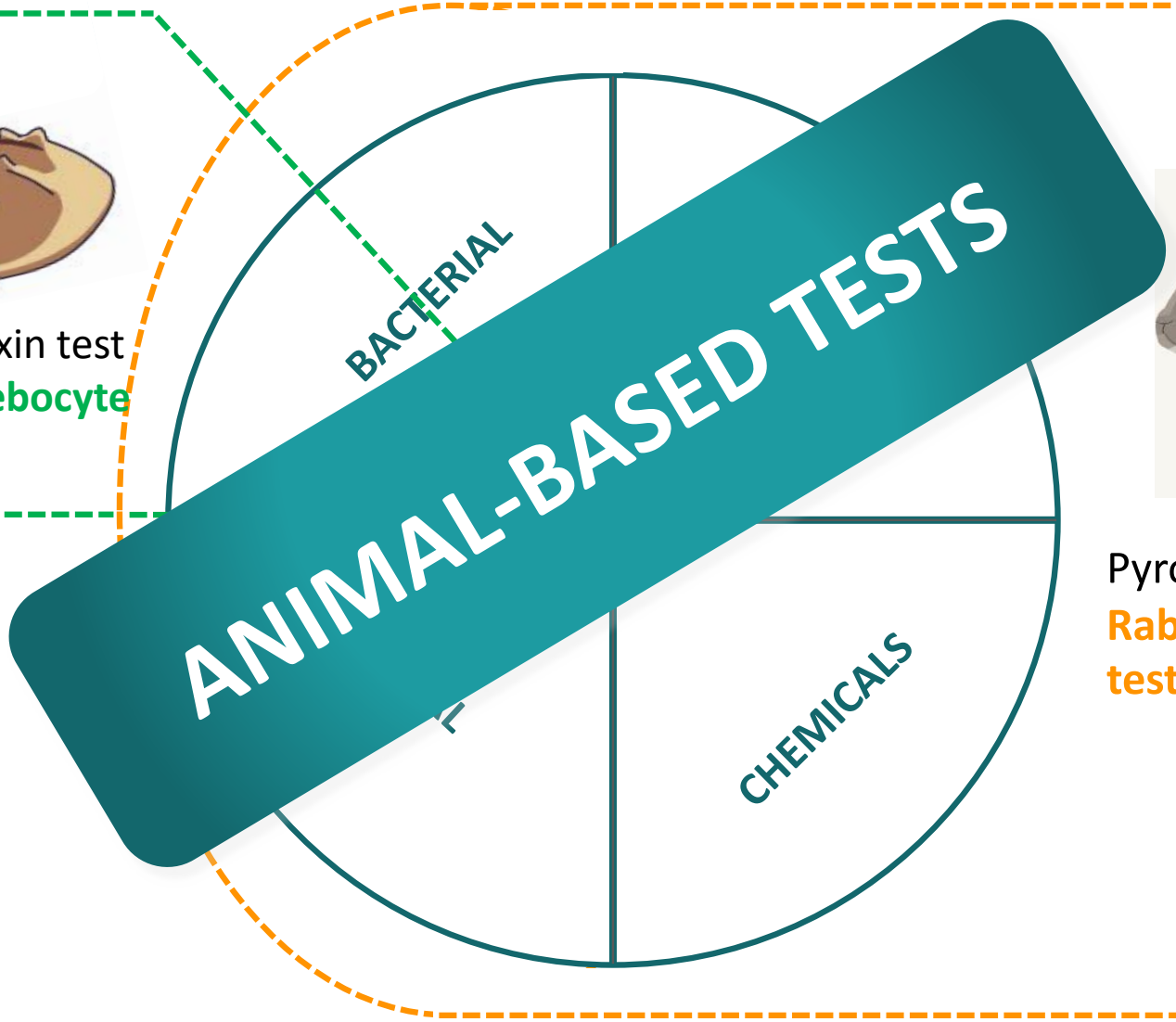
BET-Bacterial endotoxin test / LAL – Limulus amoebocyte lysate test

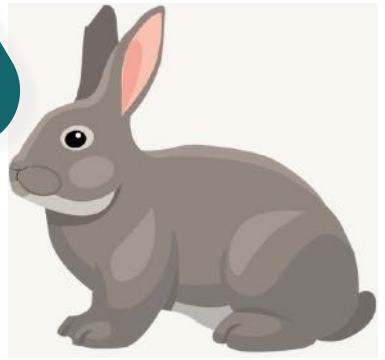
(Limit /quantitative test of endotoxin; does not detect not-endotoxin pyrogens)
[Gel-clot method; turbidimetric method and chromogenic method]

"The test is used to detect or quantify endotoxin from gram-negative bacteria using amebocyte lysate from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*)" (Chapter 2.6.14 Ph. Eur.).

ANIMAL-BASED METHODS FOR PYROGEN TESTING

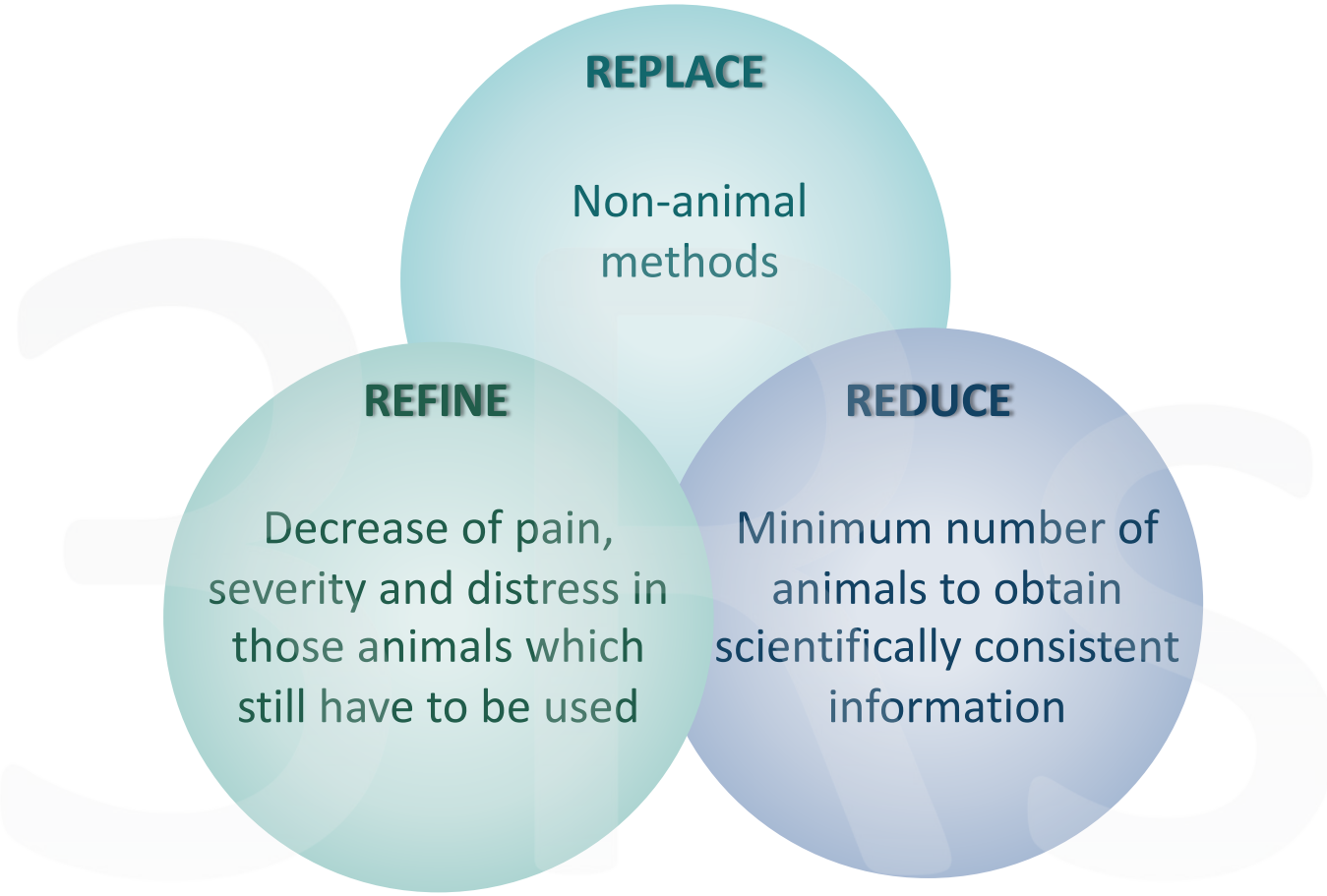
Bacterial endotoxin test by **Limulus amoebocyte lysate test - LAL**





Pyrogen testing by **Rabbit pyrogen test - RPT**

THE 3Rs PRINCIPLE

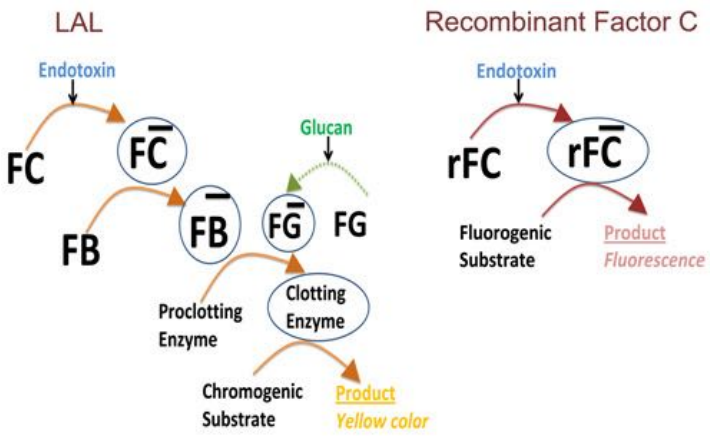


PYROGEN/ENDOTOXIN TESTS (II)

rFC- Recombinant factor C test

(Quantitative measurement of endotoxin)

The test is used to quantify endotoxin from gram-negative bacteria by mean of a non-animal-derived reagent namely Recombinant Factor C. (Chapter 2.6.32 Ph. Eur.)

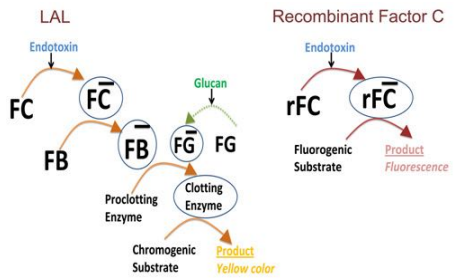


MAT- Monocyte activation test

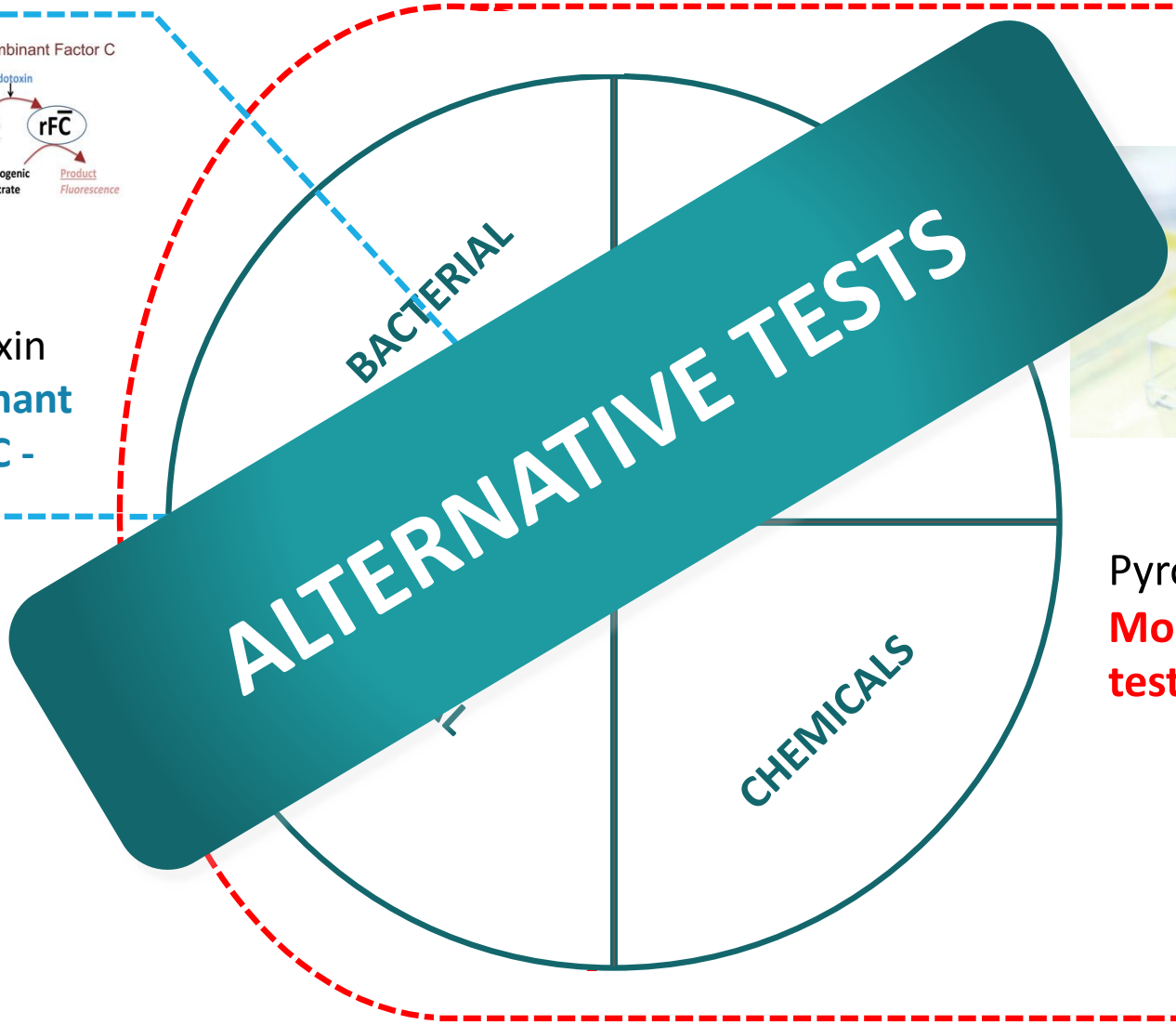
(Semi-quantitative/quantitative measurement of endotoxin and non-endotoxin pyrogens)

"The MAT is used to detect or quantify substances that activate human monocytes or monocytic cells to release endogenous mediators such as pro-inflammatory cytokines, for example TNF-α, IL-1β and IL-6. These cytokines have a role in fever pathogenesis. Consequently, the MAT will detect the presence of pyrogens in the test sample." (Chapter 2.6.30 Ph. Eur.).

ALTERNATIVE METHODS FOR PYROGEN TESTING



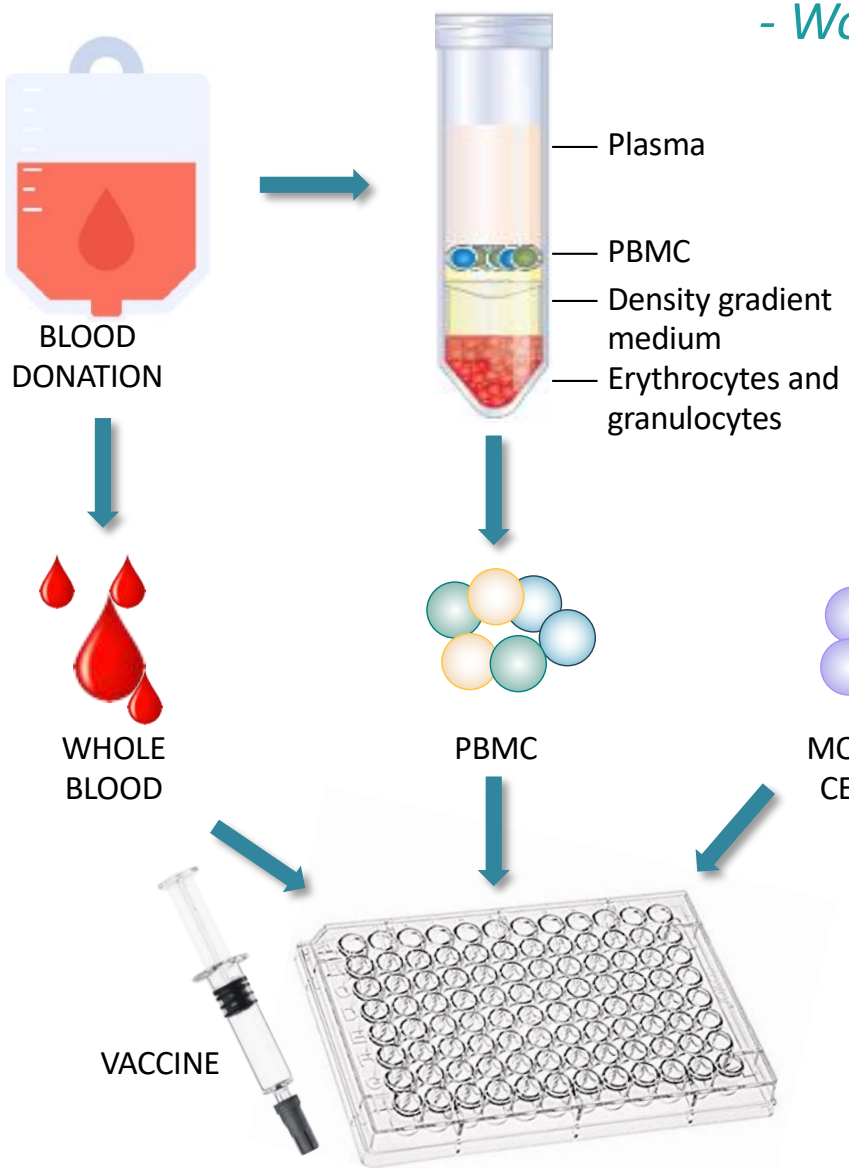
Bacterial endotoxin test by **Recombinant factor C test - rFC -**



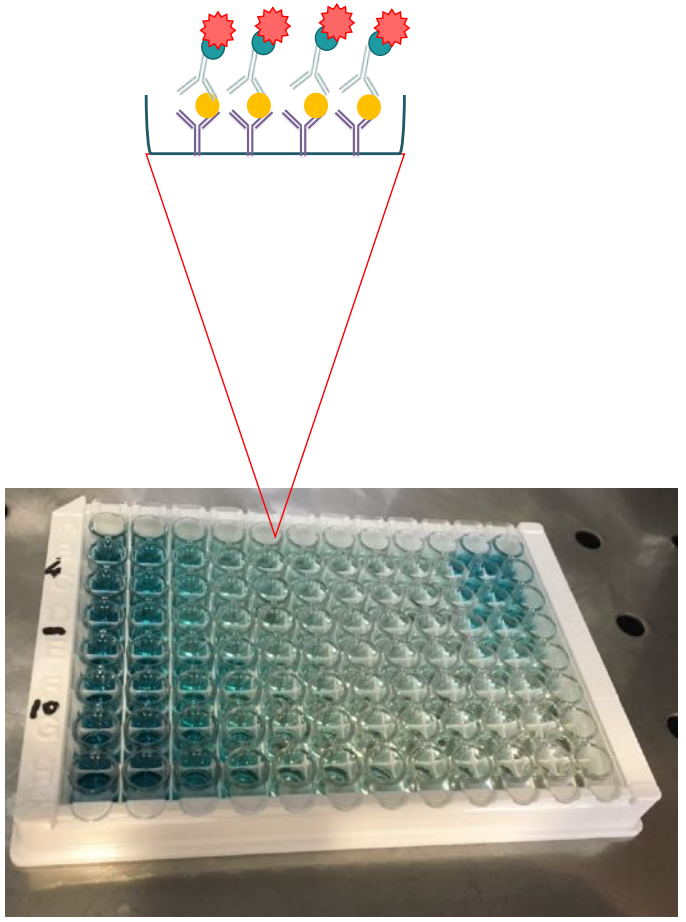
Pyrogen testing by **Monocyte Activation test - MAT**

MONOCYTE ACTIVATION TEST

- Workflow -



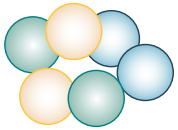
Stimulation with vaccine/reference standard endotoxin (RSE) or reference vaccine serial dilution



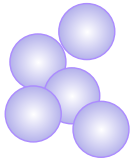
TNF-α, IL-1β or IL-6 quantification (commonly by ELISA)

CELL SOURCE FEATURES

WHOLE BLOOD



PBMCs



MONOCYTIC CELL LINES

[POLIMORFONUCLEAR AND MONONUCLEAR CELLS]	[MONONUCLEAR CELLS]	[MONO-MAC-6 AND THP1]
Donor variability	Donor variability	Very low variability
For unspecified pyrogens	For unspecified pyrogens	For known pyrogens
Presence of cytokines and Abs in plasma	Basal activation due to PBMC isolation procedures	

RABBIT PYROGEN TEST (RPT)

vs

MONOCYTE ACTIVATION TEST (MAT)

- State of art for vaccine testing -

✓**RPT:** multivalent DTwP-HepB vaccine, vaccines against HepB, rabies, pneumococcal and meningococcal polysaccharide vaccine;

✓**MAT:** *Neisseria meningitidis* group B vaccine (BEXSERO®); *Tick borne encephalitis virus* vaccine (ENCEPUR®); Salmonella vaccine (Typhim Vi® - ANSM communications to OMCL annual meeting – Sarajevo 2018)

MAT is not applied so far for the batch release of other vaccines

RABBIT PYROGEN TEST (RPT)
vs
MONOCYTE ACTIVATION TEST (MAT)
- Pros and Cons -

✓ **MAT is a non-animal alternative** to the RPT (in agreement with the 3Rs principle);

✓Since **RPT** was originally developed to test pyrogens in parenterals (administered intravenously in large volume), the method **is not appropriate for in intramuscularly or subcutaneously administered vaccines** (dilution is needed);

✓**MAT** execution (from purchase of material to data report) **is not as long as RPT**;

✓ **MAT** allows the **testing of human vaccine in human setting**;

✓ **MAT** incubation time (22 ± 2 hours) is longer than RPT (3 hours), thus **allowing the detection of delayed inflammatory response**.

2.6.30. MONOCYTE-ACTIVATION TEST

1. INTRODUCTION

The monocyte-activation test (MAT) is used to detect or quantify substances that activate human monocytes or monocytic cells to release endogenous mediators such as pro-inflammatory cytokines, for example tumour necrosis factor alpha (TNFα), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6). These cytokines have a role in fever pathogenesis. Consequently, the MAT will detect the presence of pyrogens in the test sample. The MAT is suitable, after a product-specific validation, as a replacement for the rabbit pyrogen test.

Pharmaceutical products that contain non-endotoxin pyrogenic or pro-inflammatory contaminants often show very steep or non-linear dose-response curves in comparison with endotoxin dose-response curves. Preparations that contain or may contain non-endotoxin contaminants have to be tested at a range of dilutions that includes minimum dilution.

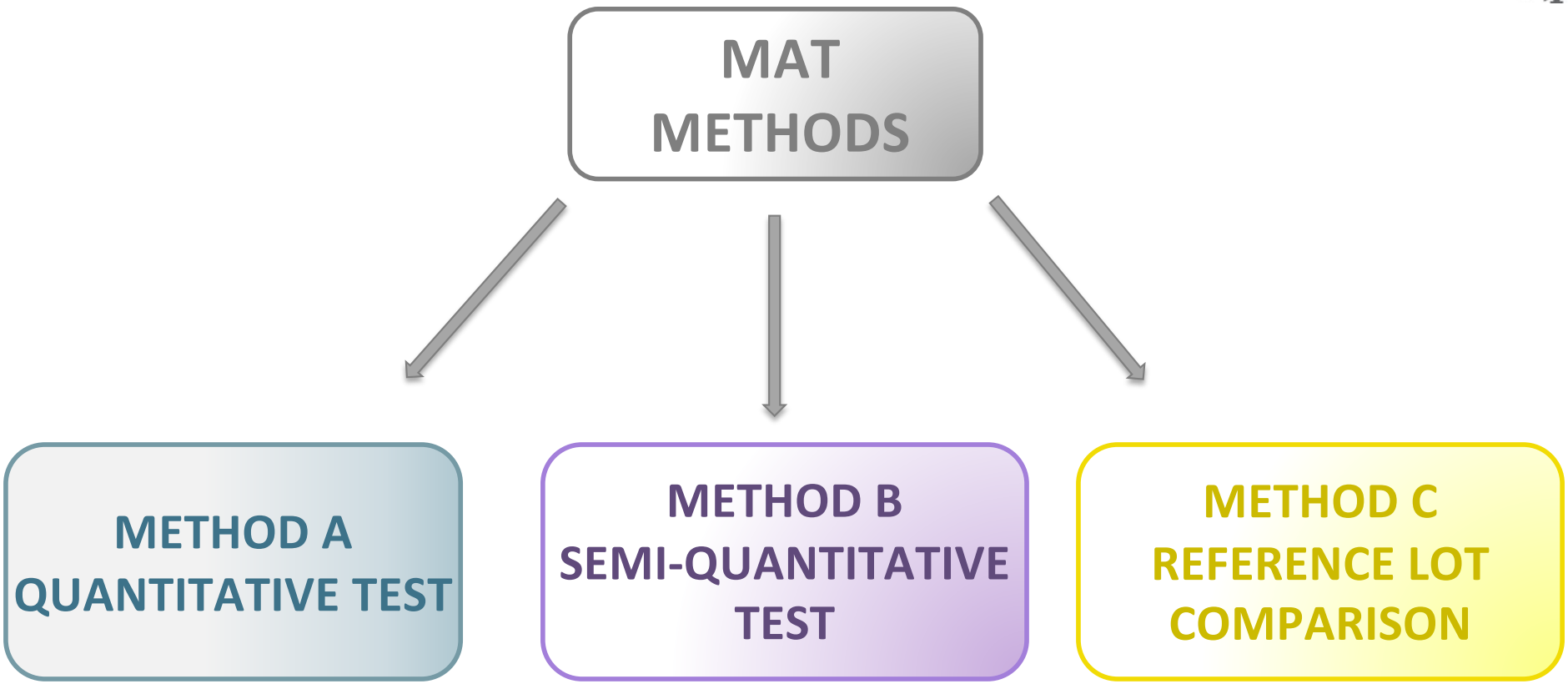
CHARACTERISTICS OF ANTIGENS AND ADJUVANTS USED IN LICENSED VACCINES

Table 1. Characteristics of adjuvants used in licensed vaccines.

Adjuvant	Composition			Major Immune Effects
(vaccines where used)	**Component**	**Origin**	**Other Uses**	
Aluminum (D, T, pertussis, IPV, hepatitis A & B, HPV, meningococcal and pneumococcal)	Aluminum as salts mixed with antigen (adsorption)	Naturally occurring present in soil, water, air	Medicines, cosmetics, food industry	Increases local inflammation, improves antigen update by APCs. Acts to increase antibody production
Virosomes (Hepatitis and influenza)	Vesicles where influenza antigens in aqueous volume are enclosed within a standard phospholipid cell membrane bilayer	Natural phospholipids, Seasonal influenza glycoproteins	None	Increases uptake by APCs. May interact with B cells leading to T-cell activation.
AS04 (Hepatitis B, HPV)	(3-deacyl-monophosphoryl lipid A) derived from LPS from *Salmonella Minnesota*, *Aluminum salts*	Natural exposure to LPS from Gram-negative bacteria occurs frequently	None	Directly stimulates TLR-4 increasing APC maturation and Th1 responses.
MF59® (Influenza-seasonal and pandemic)	Squalene	Animal source (shark liver oil). Found naturally in human tissues: adipose tissues, skin, arterial walls, skeleton, muscles, lymph nodes	Cosmetics, moisturizers	Increases APC recruitment and activation. Promotes antigen uptake and migration of cells to lymph nodes.
AS03 (Influenza-pandemic)	• Vitamin E (α-Tocopherol) • Surfactant polysorbate 80 • Squalene	• Naturally occurring in humans. • Surfactant and emulsifier • Animal source (shark liver oil). See above	• Vitamin • Used in foods, eye drops & intravenous injections • Naturally occurring. See above	Promotes local production of cytokines and recruitment of innate cells.
Thermo-reversible oil-in-water (Influenza-pandemic)	Squalene	Animal source (shark liver oil). See above	Naturally occurring. See above	Not reported
ISA51 (therapeutic vaccine NSCLC)	Mineral oil DRAKEOL 6 VR Surfactant mannide-mono-oleate	Refined mineral oil of vegetable origin	Food industry	Strongly immunogenic

D = diphtheria, T = tetanus, IPV = inactivated poliomyelitis vaccine, HPV = human papilloma virus, LPS = lipopolysaccharide, APC = antigen presenting cells, TLR = toll-like receptor, NSCLC = non-small cell lung cancer, MPL = monophosphoryl lipid A.

Alberta Di Pasquale et al. Vaccines doi:10.3390/vaccines3020320


MAT METHODS
METHOD A QUANTITATIVE TEST
METHOD B SEMI-QUANTITATIVE TEST
METHOD C REFERENCE LOT COMPARISON

METHOD A: QUANTITATIVE TEST

For products showing a parallel response respect to the dilutions of standard endotoxin. Method A involves a comparison of the preparation being examined with a standard endotoxin dose-response curve.

Table 2.6.30.-1

Solution	Solution	Added endotoxin (IU/mL)	Number of replicates
A	Test solution/*f*	None	4
B	Test solution/2 × *f*	None	4
C	Test solution/4 × *f*	None	4
AS	Test solution/*f*	Middle dose from endotoxin standard curve (R_3)	4
BS	Test solution/2 × *f*	Middle dose from endotoxin standard curve (R_3)	4
CS	Test solution/4 × *f*	Middle dose from endotoxin standard curve (R_3)	4
R_0	Pyrogen-free saline or test diluent	None (negative control)	4
R_1-R_4	Pyrogen-free saline or test diluent	4 concentrations of standard endotoxin	4 of each concentration

PASS/FAIL CRITERIA

- ✓ Criteria for endotoxin standard curve;
- ✓ The endotoxin equivalent content of the preparation being examined should be less then the contaminant limit concentration (CLC)*;
- ✓ The recovery of endotoxin in spiked test samples should fall within 50-200 %.

* The CLC is defined by considering the vaccine dose, the route of administration and the sensitivity of the set-up MAT assay.

METHOD B: SEMI-QUANTITATIVE TEST

For products/vaccines showing a not parallel response respect to the dilutions of standard endotoxin.

Method B involves a comparison of the preparation being examined with standard endotoxin.

Table 2.6.30.-2

Solution	Solution	Added endotoxin (IU/mL)	Number of replicates
A	Test solution/f	None	4
B	Test solution/f_1	None	4
C	Test solution/f_2	None	4
AS	Test solution/f	Standard endotoxin at 2 × estimated LOD for the test system	4
BS	Test solution/f_1	Standard endotoxin at 2 × estimated LOD for the test system	4
CS	Test solution/f_2	Standard endotoxin at 2 × estimated LOD for the test system	4
R_0	Pyrogen-free saline or test diluent	None (negative control)	4
R_1	Pyrogen-free saline or test diluent	Standard endotoxin at 0.5 × estimated LOD for the test system	4
R_2	Pyrogen-free saline or test diluent	Standard endotoxin at 1 × estimated LOD for the test system	4
R_3	Pyrogen-free saline or test diluent	Standard endotoxin at 2 × estimated LOD for the test system	4
R_4	Pyrogen-free saline or test diluent	Standard endotoxin at 4 × estimated LOD for the test system	4

PASS/FAIL CRITERIA

- ✓ The endotoxin equivalent content of the preparation being examined should be less then the CLC;
- ✓ The response to solution R2 should be higher then an established cut-off value;
- ✓ To determine spike-in recovery, the mean response of the spiked solution is compared with the mean response to R3 (should fall within 50-200 %).

METHOD C: REFERENCE LOT COMPARISON

Developed in order to address extreme donor variability in response to certain product containing a certain level of endotoxin and/or non-endotoxin pyrogens.

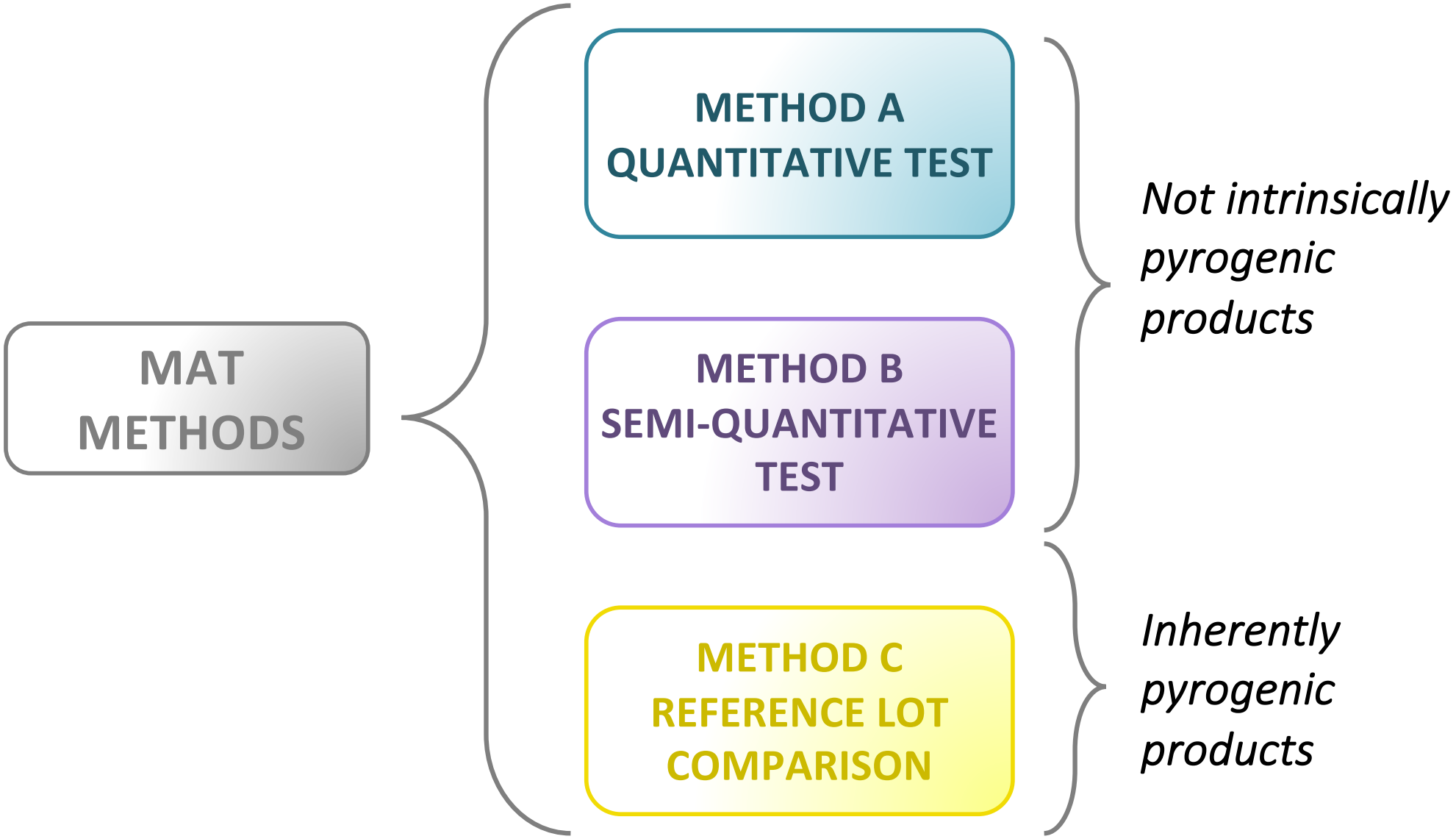
Method C involves a comparison of the preparation being examined with a validated reference lot of that preparation. The type of analysis selected to compare the two is to be justified and validated for each product and is to include assay validity criteria.

Table 2.6.30.-3

Solution	Solution/dilution factor	Number of replicates
A	Solution of reference lot/f_1	4
B	Solution of reference lot/f_2	4
C	Solution of reference lot/f_3	4
D	Solution of preparation being examined/f_1	4
E	Solution of preparation being examined/f_2	4
F	Solution of preparation being examined/f_3	4
G	Positive control (standard endotoxin)	4
R_0	Diluent (negative control)	4

PASS/FAIL CRITERIA

- ✓ Sum the mean response to solution A, B and C and the mean response to solution D, E and F. Divide the sum of D, E and F with the sum of A, B and C. The preparation being examined complies with the test if the resulting value complies with a defined acceptance criterion.


MAT METHODS
METHOD A QUANTITATIVE TEST
METHOD B SEMI-QUANTITATIVE TEST
METHOD C REFERENCE LOT COMPARISON
Not intrinsically pyrogenic products
Inherently pyrogenic products

"VACCINE BATCH TO VACCINE BATCH COMPARISON BY CONSISTENCY TESTING" PROJECT (VAC2VAC)

OBJECTIVES AND AMBITION

Proof of concept of **consistency approach** for batch release testing of established vaccines using sets of *in vitro* and analytical methods

Report on pyrogenicity assessment of human Tick-borne encephalitis virus (TBEV) vaccine (ENCEPUR®) using monocyte activation test (MAT) in human PBMC.

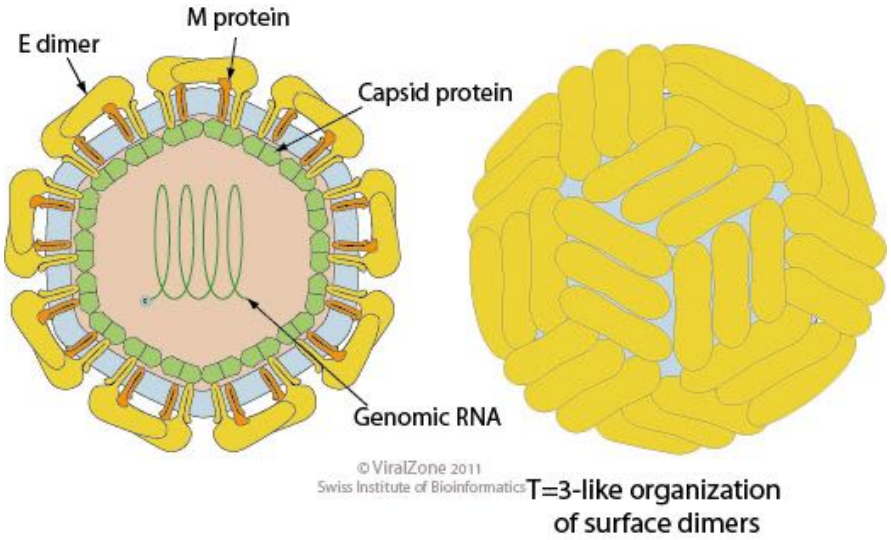
- **To replace the existing pyrogenicity test in rabbit by performing the monocyte activation test MAT assay described in the European Pharmacopoeia by using human peripheral blood mononuclear cells (h-PBMC).**
  - ✓ Develop, optimise & evaluate **non-animal methods** that cover key parameters for demonstrating batch consistency, safety and efficacy
  - ✓ (Pre-)**validate** methods and define with **regulators** guidance for regulatory approval and routine use

TICK-BORNE ENCEPHALITIS VIRUS (TBEV)

- Flavivirus
- Small enveloped virus
- Positive-sense, single-stranded RNA
- 3 structural proteins

↓

NO INTRINSIC PYROGENICITY



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TICK-BORNE ENCEPHALITIS VACCINE (INACTIVATED)

Vaccines

Vaccinum encephalitidis ixodibus advectae inactivatum

DEFINITION

Tick-borne encephalitis vaccine (inactivated) is a liquid preparation of a suitable strain of tick-borne encephalitis virus grown in cultures of chick-embryo cells or other suitable cell cultures and inactivated by a suitable, validated method.

FINAL LOT

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde, bovine serum albumin (where applicable) and pyrogens and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

IDENTIFICATION

The vaccine is shown to contain tick-borne encephalitis virus antigen by a suitable immunochemical method (*2.7.1*) using specific antibodies or by the mouse immunogenicity test described under Assay.

TESTS

Aluminium (*2.5.13*): maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (*2.4.18*): maximum of 0.1 g/l.

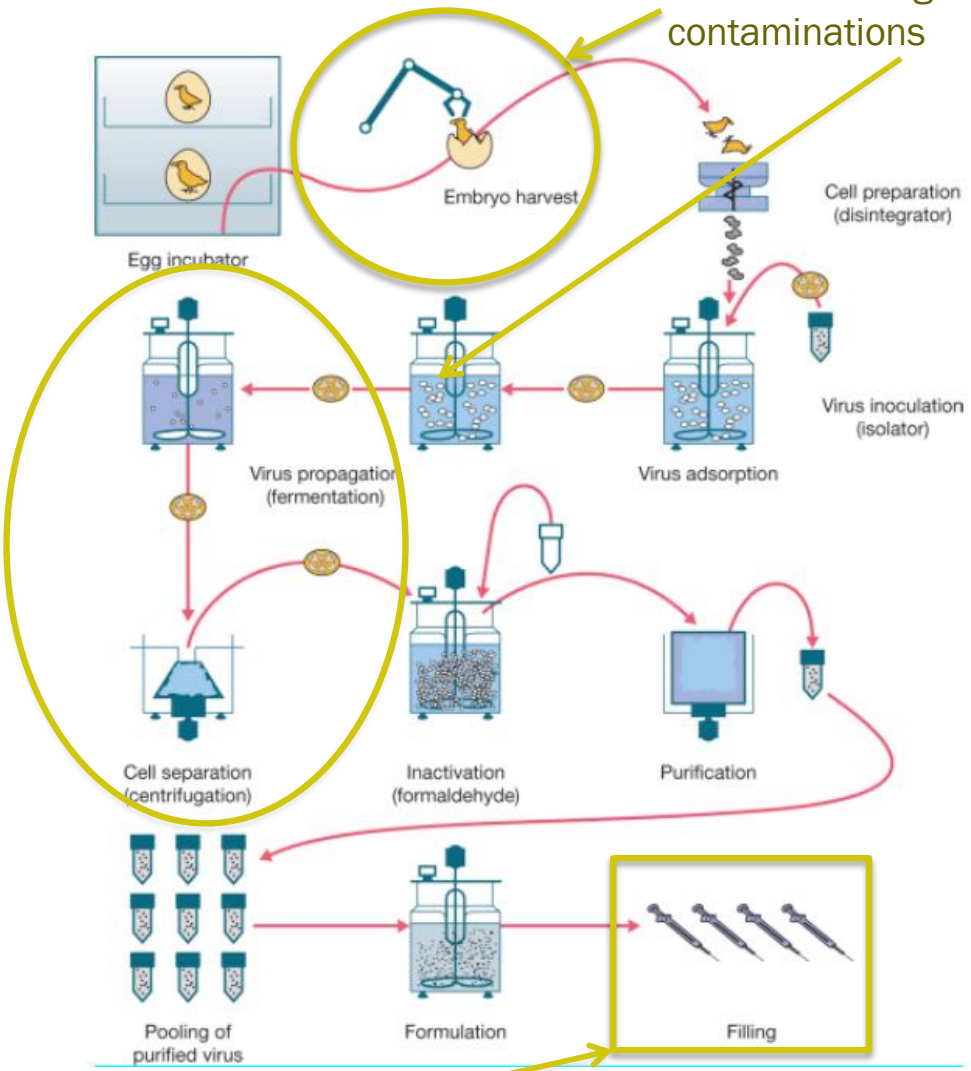
Bovine serum albumin. If bovine serum albumin has been used during production, the vaccine contains not more than 50 ng per single human dose, determined by a suitable immunochemical method (*2.7.1*).

Sterility (*2.6.1*). The vaccine complies with the test for sterility.

Pyrogens (*2.6.8*). The vaccine complies with the test for pyrogens. Inject into each rabbit, per kilogram of body mass, one dose of vaccine.

PRODUCTION PROCESS

Risk of cellular, viral, bacterial and fungal contaminations



Barrett et al., 2008 (2)

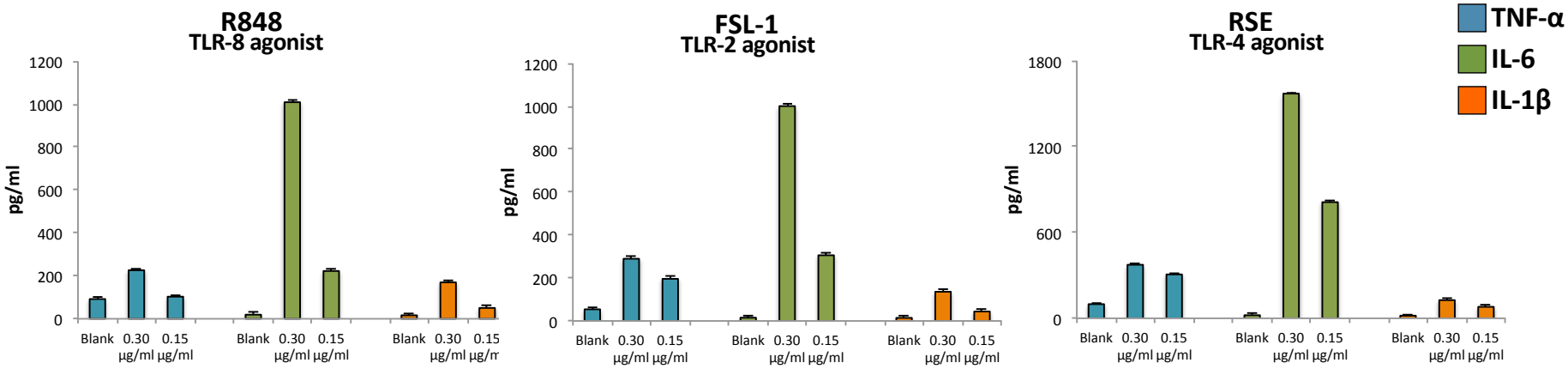
(1) *Background Document on Vaccines and Vaccination against Tick-borne Encephalitis* [Vaccine. 2011 ;29(48):8769-70]

(2) Tick borne encephalitis virus vaccines.[*Vaccines* pp. 841-856]

SETTING OF MAT CONDITIONS FOR THE TICK-BORNE ENCEPHALITIS VIRUS (TBEV) VACCINE (I)

- ✓ The MAT optimized for the TBEV vaccine was set-up by using as cell source cryopreserved **peripheral blood mononuclear cells (PBMCs)**. According to Ph.Eur., human PBMCs have been qualified:
  - PBMCs remain viable (≥ 95%) when stored at -196°C up to 18 months;
  - Reproducibility of the response to scalar doses of reference standard endotoxin (RSE) at 12 and 18 months after PBMC freezing.

- ✓ **IL-6 was chosen as read-out** providing the robust production as compared to TNF-α and IL-1β after PBMCs stimulation with RSE, and the two non-endotoxin TLR agonists R-848 and FSL-1.



SETTING OF MAT CONDITIONS FOR THE TBEV VACCINE (II)

ASSURANCE OF CRITERIA FOR ENDOTOXIN STANDARD CURVE

INTERFERENCE IN THE DETECTION SYSTEM

TEST FOR INTERFERING FACTORS

METHOD VALIDATION FOR NON-ENDOTOXIN MONOCYTE-ACTIVATING CONTAMINANTS

SETTING OF MAT CONDITIONS FOR THE TBEV VACCINE (III): PLATE LAYOUT

ACTIVE SUBSTANCE: TBEV inactivated by formaldehyde ENCEPUR®
EXCIPIENTS: Aluminum hydroxide, TRIS buffer, sucrose. Traces of tetracycline, gentamicine, neomycine and formaldehyde.

ALTEX 37(4), 000-000. doi:10.14573/altex.2002252

Research Article

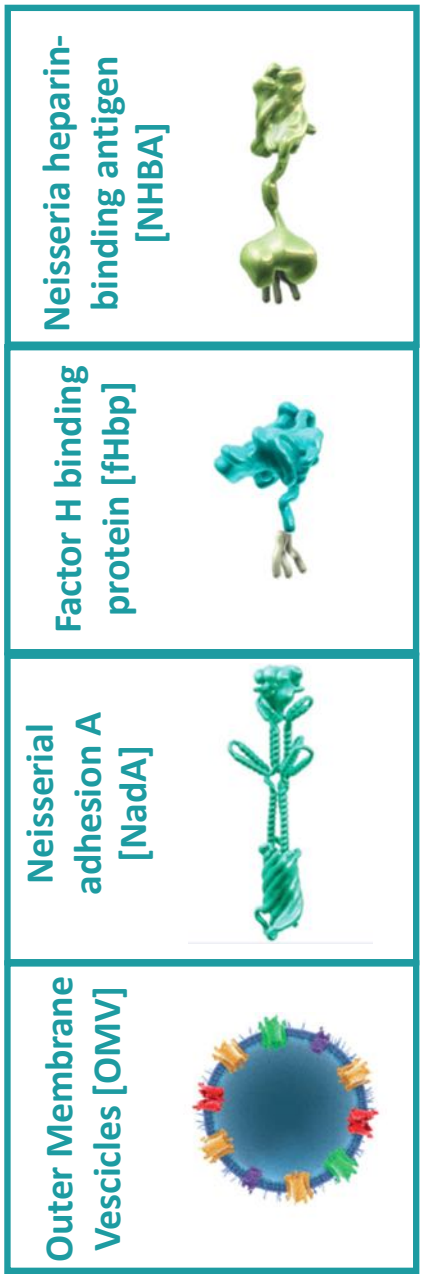
Optimization of the Monocyte Activation Test for Evaluating Pyrogenicity of Tick-Borne Encephalitis Virus Vaccine

Marilena P. Etna[1], Elena Giacomini[1], Fabiana Rizzo[1], Martina Severa[1], Daniela Ricci[1], Shahjahan Shaid[2], Denis Lambrigts[2], Sara Valentini[3], Luisa Galli Stampino[3], Liliana Alleri[3], Andrea Gaggioli[4], Christina von Hunolstein[4], Ingo Spreitzer[5] and Eliana M. Coccia[1]

[1]Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; [2]GSK, Wavre, Belgium; [3]GSK Vaccines Srl, Siena, Italy; [4]National Center for Control and Evaluation of Medicines, Istituto Superiore di Sanità, Rome, Italy; [5]Paul Ehrlich Institute, Federal Agency for Sera and Vaccines, Langen, Germany

APPLICATION OF METHOD C:
Neisseria meningitidis group B (MenB) vaccine

- ✓Recombinant fusion proteins NHBA and fHbp and recombinant protein NadA of *MenB*; *MenB* outer membrane vesicles (OMV);
- ✓OMV contain: endotoxin, porins, peptidoglycan, muramyl peptides, lipoproteins (**highly pyrogenic**);
- ✓RPT resulted not suitable to test the *MenB* vaccine;
- ✓RPT was originally developed to test pyrogens in parenterals administered intravenously in large volume therefore, the method is not appropriated for testing pyrogens in intramuscularly or subcutaneously administered vaccines (extensive dilutions are needed);
- ✓First application of MAT to a vaccine;
- ✓Reference Lot Comparison Test (**Relative Pyrogen Units**).



Modified from: https://gskpro.com/en-mt/products/bexsero/mechanism-of-action/#r11
https://www.microbiologiaitalia.it/batteriologia/meglio-bexsero-oggi-che-meningococco-domani/

WHO REQUIREMENTS FOR RPT

- State of art -

PRE-QUALIFIED VACCINES	TRS N°	Stage of RPT execution
D, T, aP, wP, HepB, IPV, Hib single or combined	980/Annex 6/2014 978/Annex 4/2013 980/Annex 4/2012	RPT or LAL on intermediate production stage and final lot
HPV (bi-, nine- and quadri-valent)	999/Annex 4/2016	If there is interference with LAL, RPT on final lot
JE (inactivated)	963/Annex 1/2007	RPT on final lot
MenA	962/Annex 2/2011	RPT on final lot
MenAC	924/Annex 2/2004	If there is interference with LAL, RPT on final lot
MenACYW-135	594/Annex 2/ 1975	RPT on final lot
PCV	977/Annex 32013	RPT on intermediate production stage; RPT or LAL on final lot
Rabies	941/Annex 2/2007	RPT on final lot
ViCPS	840/Annex 1/1992	RPT on final lot

OTHER VACCINES	TRS N°	Stage of RPT execution
HepE	WHO/BS/2018.2348	RPT on final lot
Ebola	1011/Annex 2/2018	RPT or LAL on final lot
HFRS (inactivated)	848/Annex 2/1993	RPT on final lot
RTS (Malaria)	980/Annex 3/2014	RPT or LAL on final lot
TBEV	889/Annex 2/1997	RPT on final lot

*«**With the agreement of the competent authority, alternative methods of analysis may be used for control purposes**, provided that the methods used unable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official methods were used»* (From General Notice, Ph Eur. 10.0)

EUROPEAN NATIONAL CONTROLS LABORATORIES PERFORMING MAT

Norwegian Medicines Agency

NIBSC
Confidence in Biological Medicines
NATIONAL INSTITUTE FOR BIOLOGICAL STANDARDS AND CONTROL

ISTITVTO SVPERIORE DI SANITÀ
Istituto Superiore di Sanità

HOWEVER…

Although MAT has been successfully implemented for the batch release of Men-B and TBEV vaccines, the method is not present in the current version of vaccine specific monographs as well as in the general chapter “Vaccines for human use” of Ph. Eur..

Pharmacopoeia harmonization is not too far since MAT monograph has been implemented in China, India and Canada Pharmacopoeia.

SUMMARY AND CONCLUSIONS

- ✓ MAT is intended as a **replacement** of the rabbit pyrogen test;
- ✓ The method has been already described in the general chapter of the Ph. Eur. and therefore does not require re-validation *per se* while tests for **product (vaccine)-specific optimization** are needed;
- ✓ MAT represents a human setting for testing human vaccines;
- ✓ **MAT sensitivity could be adjusted to face the heterogenicity of vaccine formulation**: ranging from the possibility to choose between primary cell or monocytic cell to three different methods of analysis;
- ✓ To rule out the presence of endotoxin and non-endotoxin pyrogens in vaccines, the MAT could be a useful tool during development of the production process (R&D), manufacturing process or for batch release.

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