EM Coccia, DCVMN Web Seminar, February 18th, 2021
MISSION: Promotion and protection of national and international public health through research, surveillance, regulation, control, prevention, communication, counseling and training activities.

COCCIA’S TEAM INVESTIGATES:

- Immune-pathogenic mechanisms of infectious diseases and escaping strategies evolved by pathogens;
- gene expression in response to infectious agents;
- immunotherapy of infectious diseases;
- alternative experimental model to test in vitro vaccine pyrogenicity and potency.
A Human Dendritic Cell-Based In Vitro Model to Assess Mycobacterium Tuberculosis SO2 Vaccine Immunogenicity

Marilena P. Etan, Elena Giacomini, Martina Severa, Manuela Pardini, Nacho Aguilo, Carlos Martin and Eliana M. Coccia

Scientific Reports

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

Frontiers in Immunology

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

Frontiers in Cellular and Infection Microbiology

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

EM Coccia, DCVMN Web Seminar, February 18th, 2021
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

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under agreement No 115924. This Joint Undertaking receives support from European Union’s Horizon 2020 research and innovation programme and EFPIA. 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.

EM Coccia, DCVMN Web Seminar, February 18th, 2021
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*
PYROGENS

ENDOTOXIN

IMPURITIES

CHEMICALS, SMALL MOLECULES

PYROGENS

GRAM + BACTERIA

VIRUS

FUNGI PARASITES

MICOPLASMA LIPOPROTEINS

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PYROGENS: WHAT THEY TRIGGER?

Exogenous Pyrogens
bacteria, virus, fungi...

Innate Immune System
monocytes, macrophages
neutrophils, dendritic cells

Endogenous Pyrogens
TNF-α, IL-1β, IL-6

Fever

PGE₂

Hypothalamous

Testing of chemicals or biologicals, including vaccines, prior to batch release is relevant

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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?

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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

**REPLACE**

Non-animal methods

**REFINE**

Decrease of pain, severity and distress in those animals which still have to be used

**REDUCE**

Minimum number of animals to obtain scientifically consistent information

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**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

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**MONOCYTE ACTIVATION TEST**

- **Workflow** -

**BLOOD DONATION**

Plasma

PBMC

Density gradient medium

Erythrocytes and granulocytes

**WHOLE BLOOD**

PBMC

**VACCINE**

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

**MONOCYTIC CELL LINES**

22 h ± 1 h

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

**Capture Antibody**

**Antigen**

**Detection Antibody**

**Streptavidin-HRP**

**Substrate**

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## CELL SOURCE FEATURES

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Donor Variability</th>
<th>Presence of Cytokines and Abs in Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>Donor variability</td>
<td>Basal activation due to PBMC isolation procedures</td>
</tr>
<tr>
<td>PBMCs</td>
<td></td>
<td>Presence of cytokines and Abs in plasma</td>
</tr>
<tr>
<td>Monocytic Cell Lines</td>
<td>Very low variability</td>
<td>For known pyrogens</td>
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**Cell Source Features**

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<td>Monocytic Cell Lines</td>
<td>Very low variability</td>
<td>For known pyrogens</td>
</tr>
</tbody>
</table>

**Notes:**

- For unspecified pyrogens:
  - Presence of cytokines and Abs in plasma
- Basal activation due to PBMC isolation procedures

**MP Etna, DCVMN Web Seminar, February 18th, 2021**
**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**
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.
onset of encephalitis in children. Pertussis vaccination coverage dropped precipitously in some countries (such as the United Kingdom), and vaccination against pertussis was halted in others (Sweden), resulting in national pertussis outbreaks of a magnitude not seen for decades [7,8]. The response of the scientific community was to search for purified antigens (or subunits) capable of inducing a protective immune response and with improved reactogenicity profiles [9]. The resulting acellular pertussis vaccines containing between one and five purified antigens demonstrated lower rates of local and systemic reactions after vaccination compared with whole-cell vaccines [10]. Nevertheless, the duration of immunity induced by acellular pertussis vaccines appears to be shorter than expected [11–13], underlining the need for regular booster doses in older children as well as in adolescents, adults, and the elderly. At the same time, the search continues for improved pertussis vaccines that induce more durable protection [14].

Other new vaccine approaches were developed to address a range of technical and implementation-related challenges. For example, recombinant technologies allowed the production of vaccines for pathogens unable to be grown in vitro. Pathogens with multiple disease-causing strains/serogroups required methods to combine multiple antigens into a single vial. Efforts were also made to improve vaccine acceptance and coverage using complex multi-valent vaccines targeting multiple different diseases in the same injection.

While vaccines containing a limited number of purified antigens generally have improved safety profiles compared with live-attenuated and whole-pathogen vaccines, they are also often less immunogenic due to the removal of pathogenic features of the organism (Figure 1) [15].

**Table 1. Characteristics of adjuvants used in licensed vaccines.**

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Component</th>
<th>Origin</th>
<th>Other Uses</th>
<th>Major Immune Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum (D, T, pertussis, IPV, hepatitis A &amp; B, HPV, meningococcal and pneumococcal)</td>
<td>Aluminum as salts mixed with antigen (adsorption)</td>
<td>Naturally occurring present in soil, water, air</td>
<td>Medicines, cosmetics, food industry</td>
<td>Increases local inflammation, improves antigen uptake by APCs. Acts to increase antibody production</td>
</tr>
<tr>
<td>Virosomes (Hepatitis and influenza)</td>
<td>Vesicles where influenza antigens in aqueous volume are enclosed within a standard phospholipid cell membrane bilayer</td>
<td>Natural phospholipids, Seasonal influenza glycoproteins</td>
<td>None</td>
<td>Increases uptake by APCs. May interact with B cells leading to T-cell activation.</td>
</tr>
<tr>
<td>AS04 (Hepatitis B, HPV)</td>
<td>(3-deacyl-monophosphoryl lipid A) derived from LPS from <em>Salmonella Minnesota, Aluminum salts</em></td>
<td>Natural exposure to LPS from Gram-negative bacteria occurs frequently</td>
<td>None</td>
<td>Directly stimulates TLR-4 increasing APC maturation and Th1 responses.</td>
</tr>
<tr>
<td>MF59* (Influenza-seasonal and pandemic)</td>
<td>Squalene</td>
<td>Animal source (shark liver oil). Found naturally in human tissues: adipose tissues, skin, arterial walls, skeleton, muscles, lymph nodes</td>
<td>Cosmetics, moisturizers</td>
<td>Increases APC recruitment and activation. Promotes antigen uptake and migration of cells to lymph nodes.</td>
</tr>
</tbody>
</table>
| 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 mamiide-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.

MAT METHODS

METHOD A
QUANTITATIVE TEST

METHOD B
SEMI-QUANTITATIVE TEST

METHOD C
REFERENCE LOT COMPARISON

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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>
<thead>
<tr>
<th>Solution</th>
<th>Solution</th>
<th>Added endotoxin (IU/mL)</th>
<th>Number of replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Test solution/f</td>
<td>None</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>Test solution/2×f</td>
<td>None</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>Test solution/4×f</td>
<td>None</td>
<td>4</td>
</tr>
<tr>
<td>AS</td>
<td>Test solution/f</td>
<td>Middle dose from endotoxin standard curve (R₁)</td>
<td>4</td>
</tr>
<tr>
<td>BS</td>
<td>Test solution/2×f</td>
<td>Middle dose from endotoxin standard curve (R₂)</td>
<td>4</td>
</tr>
<tr>
<td>CS</td>
<td>Test solution/4×f</td>
<td>Middle dose from endotoxin standard curve (R₄)</td>
<td>4</td>
</tr>
<tr>
<td>R₀</td>
<td>Pyrogen-free saline or test diluent</td>
<td>None (negative control)</td>
<td>4</td>
</tr>
<tr>
<td>R₁-R₄</td>
<td>Pyrogen-free saline or test diluent</td>
<td>4 concentrations of standard endotoxin</td>
<td>4 of each concentration</td>
</tr>
</tbody>
</table>

PASS/FAIL CRITERIA

✓ Criteria for endotoxin standard curve;
✓ The endotoxin equivalent content of the preparation being examined should be less than 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.

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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

<table>
<thead>
<tr>
<th>Solution</th>
<th>Solution</th>
<th>Added endotoxin (IU/mL)</th>
<th>Number of replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Test solution/f</td>
<td>None</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>Test solution/f</td>
<td>None</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>Test solution/f</td>
<td>None</td>
<td>4</td>
</tr>
<tr>
<td>AS</td>
<td>Test solution/f</td>
<td>Standard endotoxin at 2 × estimated LOD for the test system</td>
<td>4</td>
</tr>
<tr>
<td>BS</td>
<td>Test solution/f</td>
<td>Standard endotoxin at 2 × estimated LOD for the test system</td>
<td>4</td>
</tr>
<tr>
<td>CS</td>
<td>Test solution/f</td>
<td>Standard endotoxin at 2 × estimated LOD for the test system</td>
<td>4</td>
</tr>
<tr>
<td>R1</td>
<td>Pyrogen-free saline or test diluent</td>
<td>None (negative control)</td>
<td>4</td>
</tr>
<tr>
<td>R2</td>
<td>Pyrogen-free saline or test diluent</td>
<td>Standard endotoxin at 0.5 × estimated LOD for the test system</td>
<td>4</td>
</tr>
<tr>
<td>R3</td>
<td>Pyrogen-free saline or test diluent</td>
<td>Standard endotoxin at 1 × estimated LOD for the test system</td>
<td>4</td>
</tr>
<tr>
<td>R4</td>
<td>Pyrogen-free saline or test diluent</td>
<td>Standard endotoxin at 2 × estimated LOD for the test system</td>
<td>4</td>
</tr>
<tr>
<td>R5</td>
<td>Pyrogen-free saline or test diluent</td>
<td>Standard endotoxin at 4 × estimated LOD for the test system</td>
<td>4</td>
</tr>
</tbody>
</table>

PASS/FAIL CRITERIA

- The endotoxin equivalent content of the preparation being examined should be less than the CLC;
- The response to solution R2 should be higher than 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%).

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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

<table>
<thead>
<tr>
<th>Solution</th>
<th>Solution/dilution factor</th>
<th>Number of replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Solution of reference lot/f&lt;sub&gt;i&lt;/sub&gt;</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>Solution of reference lot/f&lt;sub&gt;i&lt;/sub&gt;</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>Solution of reference lot/f&lt;sub&gt;i&lt;/sub&gt;</td>
<td>4</td>
</tr>
<tr>
<td>D</td>
<td>Solution of preparation being examined/f&lt;sub&gt;i&lt;/sub&gt;</td>
<td>4</td>
</tr>
<tr>
<td>E</td>
<td>Solution of preparation being examined/f&lt;sub&gt;i&lt;/sub&gt;</td>
<td>4</td>
</tr>
<tr>
<td>F</td>
<td>Solution of preparation being examined/f&lt;sub&gt;i&lt;/sub&gt;</td>
<td>4</td>
</tr>
<tr>
<td>G</td>
<td>Positive control (standard endotoxin)</td>
<td>4</td>
</tr>
<tr>
<td>R&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Diluent (negative control)</td>
<td>4</td>
</tr>
</tbody>
</table>

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.
METHOD A
QUANTITATIVE TEST

METHOD B
SEMI-QUANTITATIVE TEST

METHOD C
REFERENCE LOT COMPARISON

MAT METHODS

Not intrinsically pyrogenic products

Inherently pyrogenic products

MP Etna, DCVMN Web Seminar, February 18th, 2021
“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.

- Develop, optimise & evaluate non-animal methods that cover key parameters for demonstrating batch consistency, safety and efficacy.
- 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).
- (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
**TICK-BORNE ENCEPHALITIS VACCINE (INACTIVATED)**

**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]
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

MP Etna, DCVMN Web Seminar, February 18th, 2021
ACTIVE SUBSTANCE: TBEV inactivated by formaldehyde ENCEPUR®
EXCIPIENTS: Aluminum hydroxide, TRIS buffer, sucrose. Traces of tetracycline, gentamicine, neomycine and formaldehyde.

Research Article

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

Marilena P. Etna¹, Elena Giacomini¹, Fabiana Rizzo¹, Martina Severa¹, Daniela Ricci¹, Shahjahan Shaid², Denis Lambrigts², Sara Valentini³, Luisa Galli Stampino³, Liliana Alleri³, Andrea Gaggioli⁴, Christina von Hunolstein⁴, Ingo Spreitzer⁵ and Eliana M. Coccia¹

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

ALTEX 37(4), 000-000. doi:10.14573/altex.2002252
APPLICABLE 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*).
WHO REQUIREMENTS FOR RPT
- State of art -

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

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

«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

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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