Application of Monocyte Activation Test for the Assessment of Tick-Borne Encephalitis Virus Vaccine Pyrogenicity

Status of global implementation of MAT for biologicals. Product Specific Approaches and Regulatory Alignment

AFSA Workshop

September 25th, 2024

Marilena P. Etna, PhD
Istituto Superiore di Sanità, Rome (Italy)
Department of Infectious Diseases

ITALIAN NATIONAL INSTITUTE OF HEALTH [ISS]

DIPARTIMENTO MALATTIE INFETTIVE

MAT UNIT

Eliana M. Coccia
(Head of MAT Unit)
Marilena P. Etna
(Expert of MAT Unit)
Ramona Ilari
(Analyst)
Simone Trichei
(Analyst)

vac2vac

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

VAC2VAC - Vaccine batch to vaccine batch comparison by consistency testing

One of the objective of VAC2VAC project was the development and the optimization of cellular assays based on analysis of human tick-borne encephalitis virus (TBEV) vaccine-induced activation of primary APC.

↓

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 (PBMC).

01/2019:1375

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. The assay also serves to identify the vaccine.

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 0.1 g/L.

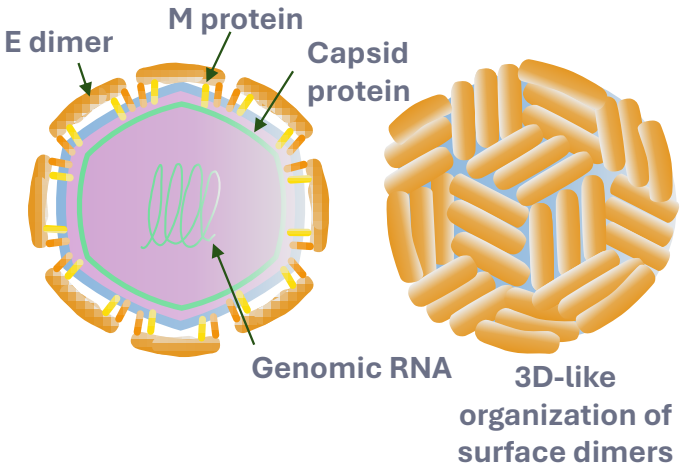
Bovine serum albumin: maximum 50 ng per single human dose, determined by a suitable immunochemical method (*2.7.1*), if bovine serum albumin has been used during production.

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, 1 dose of vaccine.

VACCINE ACTIVE SUBSTANCE

TICK-BORNE ENCEPHALITIS VIRUS

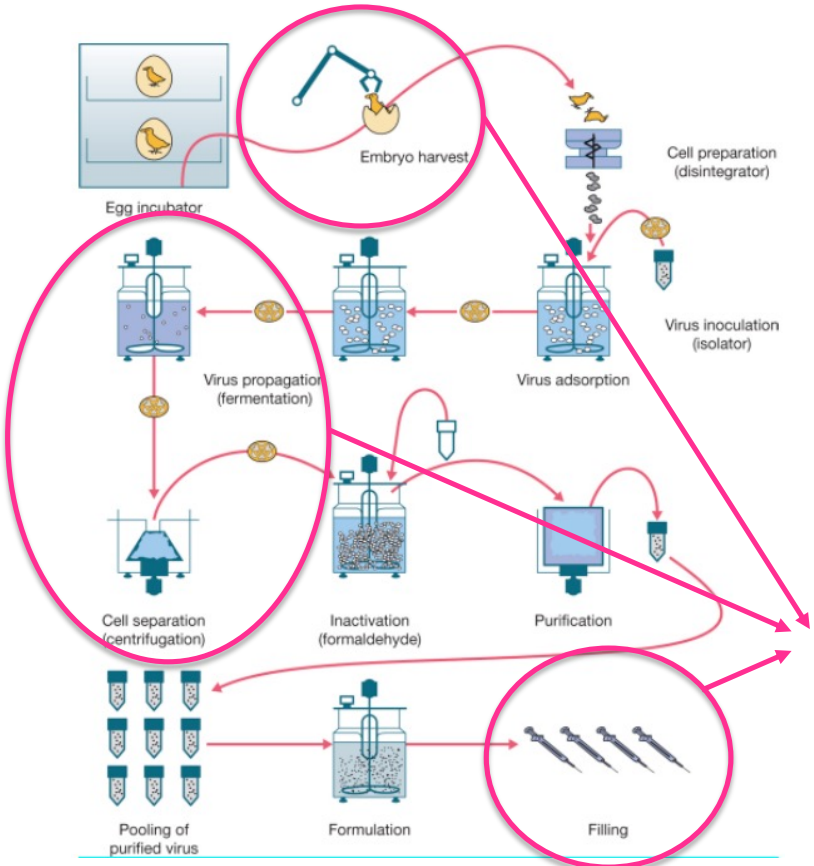


- Flavivirus
- Small enveloped virus
- Positive-sense, single-stranded RNA
- 3 structural proteins

NO INTRINSIC PYROGENICITY

TBEV vaccine production process

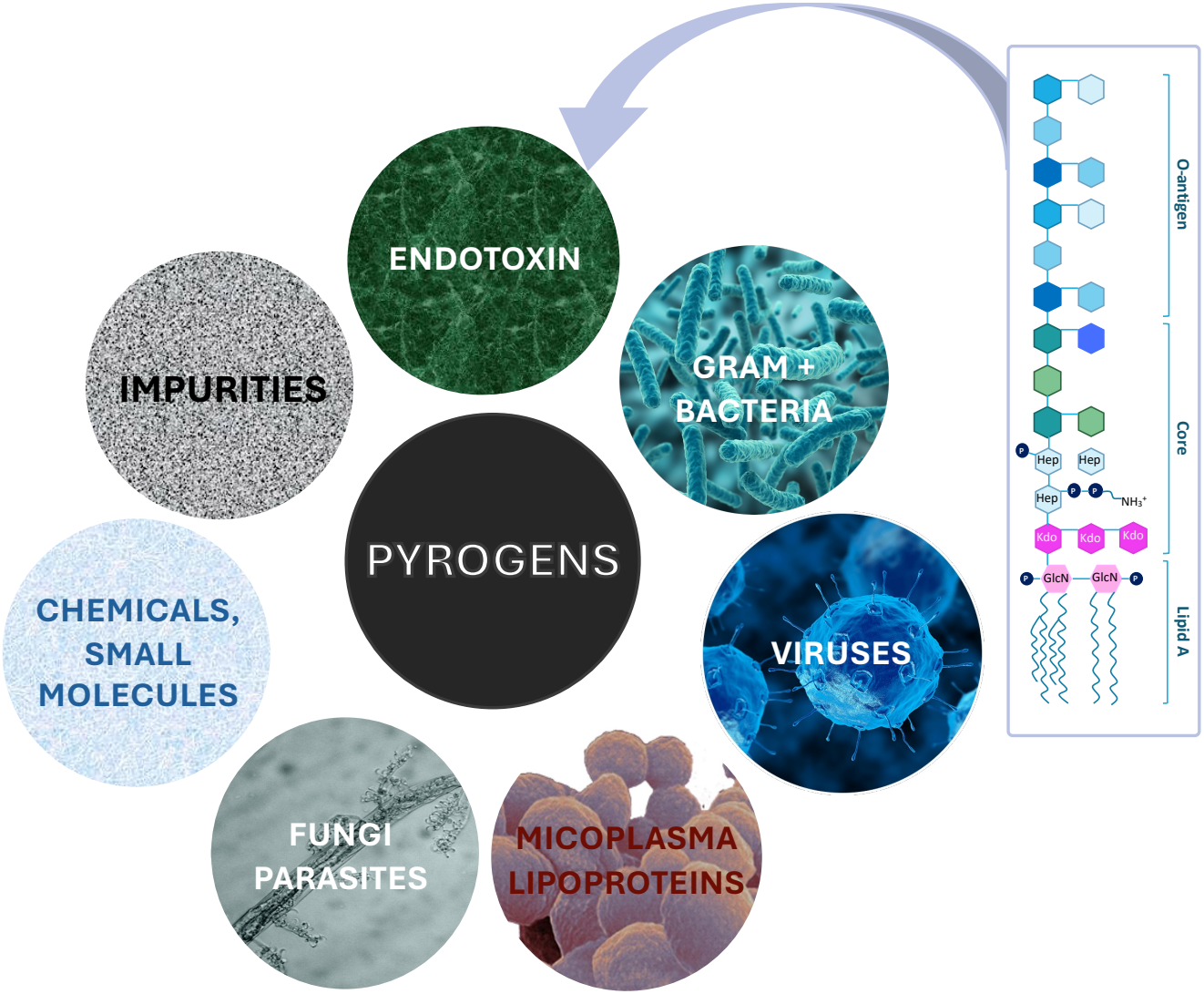
(1) *Background Document on Vaccines and Vaccination against Tick-borne Encephalitis* [Vaccine. 2011 ;29(48):8769-70]
(2) Tick borne encephalitis virus vaccines.[*Vaccines* pp. 841-856]



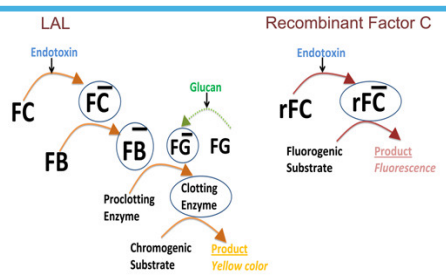
Barrett et al., 2008 (2)

TBEV (active substance) is cultivated in chicken embryo cells, clarified by centrifugation, inactivated with formalin and then purified to produce the vaccine virus stock. Pools of different purified virus stock were formulated with aluminum hydroxide (adjuvant)

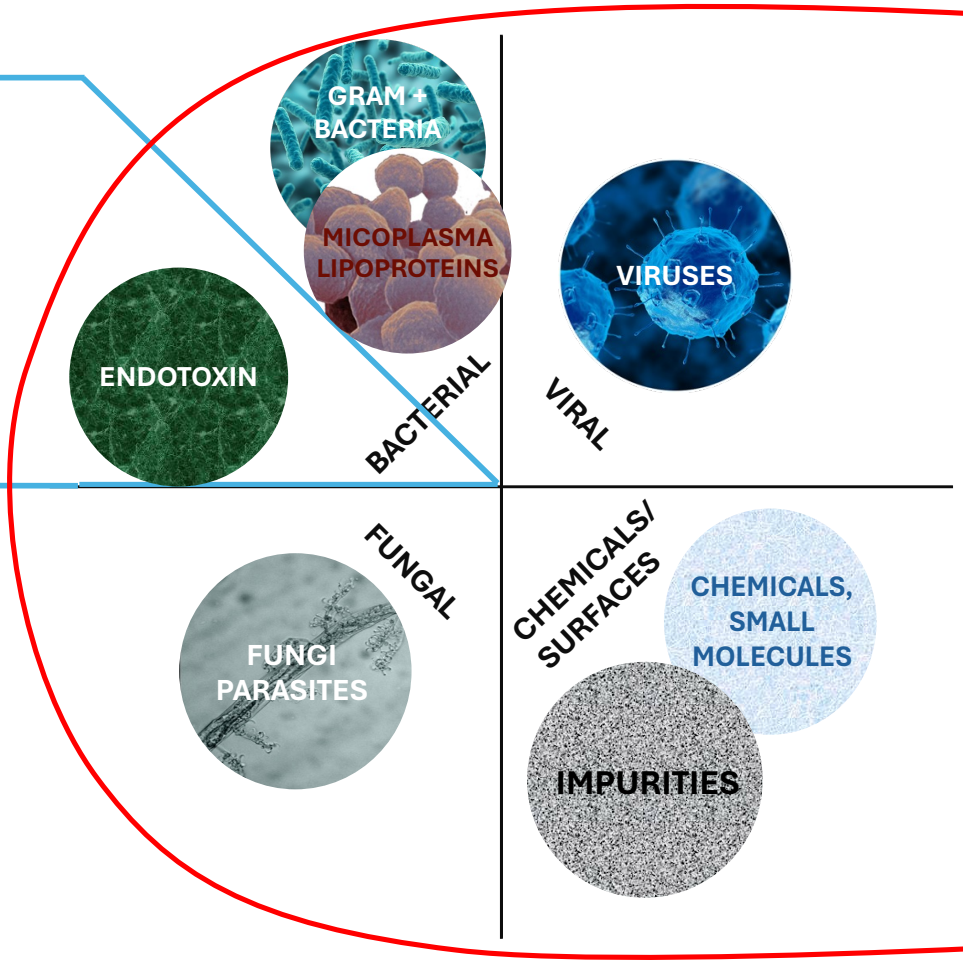
Embryo harvest from chicken eggs or the virus propagation could entail the risk of bacterial, viral or cellular contaminants entering the final product


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

Non-Animal Methods for endotoxin and pyrogen testing



Bacterial endotoxin test by
Recombinant factor C test [rFC]



Pyrogen testing by
Monocyte Activation test [MAT]

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

ALTEX 37(4), 532-544. doi:10.14573/altex.2002252

Qualification of cell source (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 by assessing:

- PBMC viability
- Reproducibility of the response to scalar doses of reference standard endotoxin (RSE)

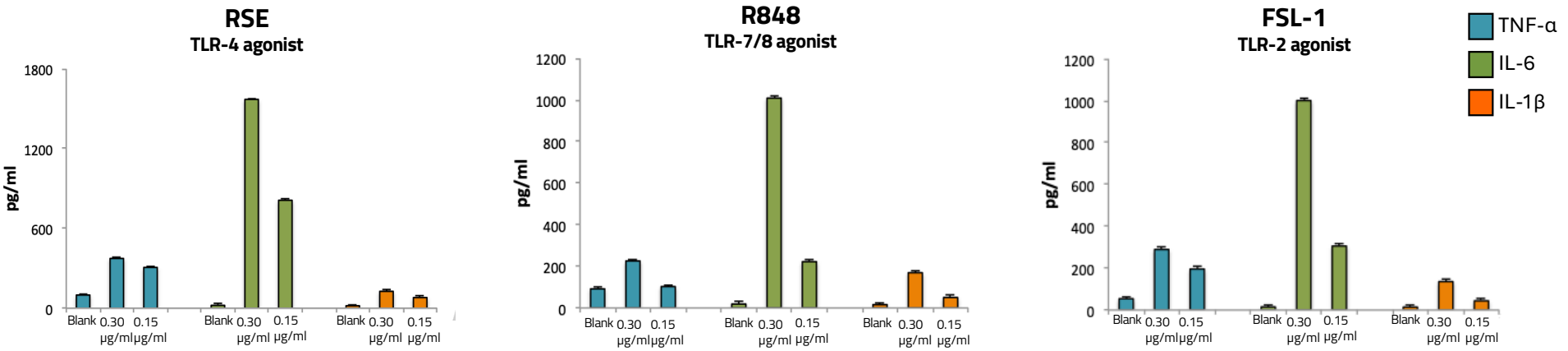
Qualification of cell source (II)

	Cell viability (%)			RSE 0.2 EU/mL (IL-6 pg/mL)			RSE 0.4 EU/mL (IL-6 pg/mL)		
	6 months	12 months	18 months	6 months	12 months	18 months	6 months	12 months	18 months
PBMC_Donor 1	89.0	93.0	93.0	566	480	400	1200	1324	896
PBMC_Donor 2	91.2	95.5	95.5	500	455	475	1000	1000	1000
PBMC_Donor 3	95.0	92.0	91.0	410	370	430	1200	813	1200
PBMC_Donor 4	91.0	93.7	90.0	502	500	394	1213	1000	924

Etna MP et al., **ALTEX 37(4), 532-544**. doi:10.14573/altex.2002252

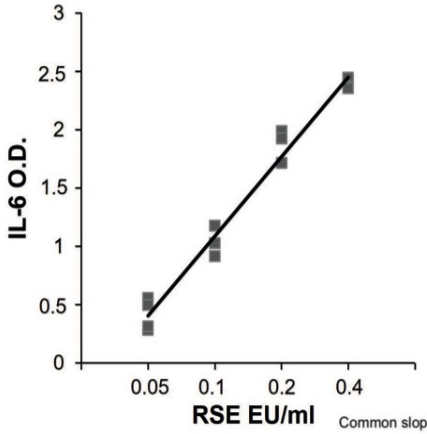
- PBMCs remain viable (≥ 90%) when stored at -196°C up to 18 months
- Response to scalar doses of RSE is reproducible and stable up to 12 and 18 months after PBMC freezing

Choice of the Read-out



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

Preparatory test: Assurance of criteria for endotoxin standard curve



Model: Parallel lines
Design: Completely randomised
Transformation: y' = y
Variance: Observed residuals

Common slope(factor) = 0.981682 (0.919868 to 1.04350)
Correlation | r |: 0.987971

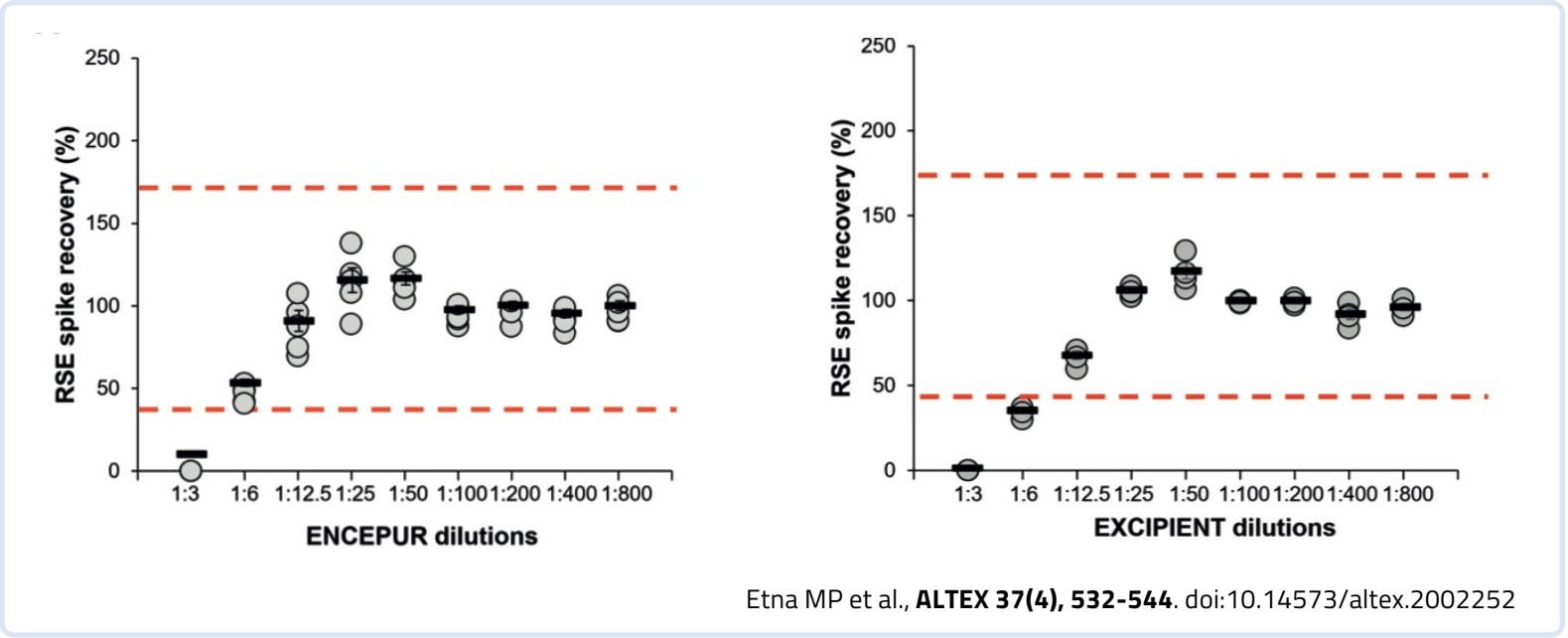
Source of variation	Degrees of freedom	Sum of squares	Mean square	F-ratio	Probability
* Regression	1	9.26024	9.26024	801.166	0.000 (***)
* Non-linearity	2	0.0881724	0.0440862	3.814	0.052
Treatments	3	9.34842	3.11614	269.598	0.000 (***)
Residual error	12	0.138702	0.0115585		
Total	15	9.48712	0.632475		

Etna MP et al., **ALTEX 37(4), 532-544**. doi:10.14573/altex.2002252

Linear Response to different RSE doses:

- Statistically significant regression of response on Log_{10} dose of RSE ($p < 0.01$)
- Not significant deviation of RSE Log_{10} dose from linearity ($p > 0.05$)

Preparatory test: Test for interfering factors



Etna MP et al., **ALTEX 37(4), 532-544**. doi:10.14573/altex.2002252

In vaccine dilutions starting from 1:12.5 **RSE recovery** was **within** the permitted range of **50-200%**

Preparatory test: Interference in the Detection System

Standard		Standard+V1		Standard+V2		Standard+V3		Standard+V4		Standard+V5	
pg/mL	OD	OD	Interference (%)	OD	Interference (%)	OD	Interference (%)	OD	Interference (%)	OD	Interference (%)
600	2.629	2.549	-3.4%	2.507	-4.6%	2.580	-1.86%	2.415	-8.1%	2.535	-3.5%
300	1.788	1.770	-1.0%	1.818	+1.6%	1.579	-11.6%	1.719	-3.8%	1.662	-7.0%
150	1.095	1.061	-3.1%	1.024	-6.4%	0.924	-15.6%	1.001	-8.5%	0.980	-10.5%
75	0.593	0.555	-6.4%	0.565	-4.7%	0.500	-15.6%	0.562	-5.2%	0.526	-11.2%
37.5	0.294	0.240	-18.3%	0.291	-1.0%	0.244	-17.0%	0.276	-6.1%	0.265	-9.8%
18.8	0.140	0.131	-6.4%	0.130	-7.1%	0.111	-20.0%	0.116	-17.1%	0.120	-14.2%
9.38	0.076	0.073	-3.9%	0.062	-18.4%	0.068	-10.5%	0.068	-10.5%	0.066	-13.1%

V1= 1µg/ml [1:3]; V2= 0.5 µg/ml [1:6]; V3= 0.25 µg/ml [1:12.5]; V4= 0.125 µg/ml [1:25]; V5= 0.0625 µg/ml [1:50]

Etna MP et al., **ALTEX 37(4), 532-544**. doi:10.14573/altex.2002252

TBEV vaccine does not interfere with the ELISA procedure since variations in OD values fall within +/- 20%

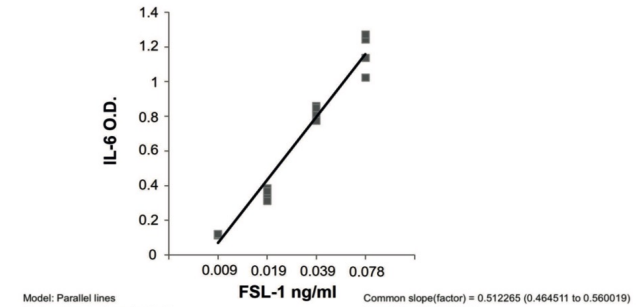
Preparatory test: Method validation for non-endotoxin monocytes-activating contaminants

- To verify whether the experimental conditions employed for the assay ensure a **linear response to different doses of 2 NEPs** (non-endotoxin pyrogens)

- To assess **whether the product** to be tested (i.e., the vaccine) **interferes with the detection of NEPs**

Preparatory test: Method validation for non-endotoxin monocytes-activating contaminants

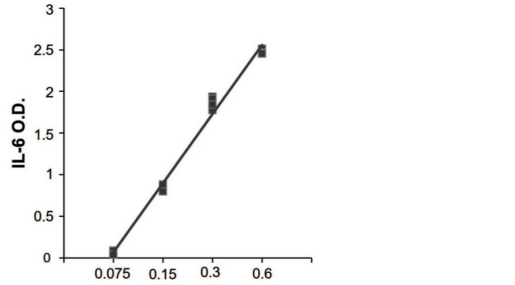
- MAT optimized for the TBEV vaccine shows a linear response to NEPs



Model: Parallel lines
Design: Completely randomised
Transformation: y' = y
Variance: Observed residuals

Common slope(factor) = 0.512265 (0.464511 to 0.560019)
Correlation | r |: 0.977391

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-ratio	Probability
Regression	1	2.52157	2.52157	365.538	0.000 (***)
Non-linearity	2	0.0352286	0.0176143	2.553	0.119
Treatments	3	2.55679	0.852265	123.548	0.000 (***)
Residual error	12	0.0827787	0.00689823		
Total	15	2.63957	0.175971		

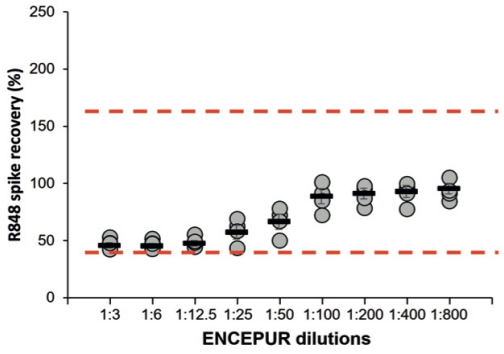


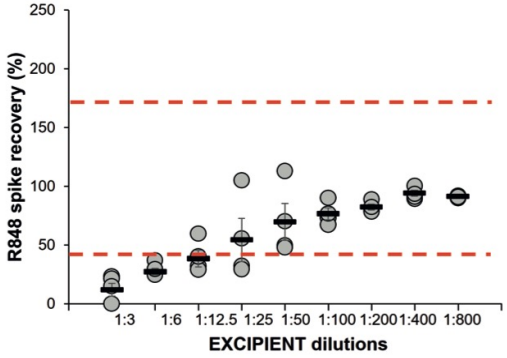
Model: Parallel lines
Design: Completely randomised
Transformation: y' = y
Variance: Observed residuals

Common slope(factor) = 1.09410 (1.02826 to 1.15995)
Correlation | r |: 0.989451

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-ratio	Probability
Regression	1	11.5027	11.5027	877.090	0.000 (***)
Non-linearity	2	0.0891984	0.0445992	3.401	0.068
Treatments	3	11.5919	3.86395	294.631	0.000 (***)
Residual error	12	0.157375	0.0131146		
Total	15	11.7492	0.783282		

Etna MP et al., **ALTEX 37(4), 532-544**. doi:10.14573/altex.2002252





Etna MP et al., **ALTEX 37(4), 532-544**. doi:10.14573/altex.2002252

- TBEV vaccine does not interferes with NEP detection starting from 1:100

Determination of LOD, AS, MVD and CLC for MAT optimized for the TBEV vaccine

TLR agonist	LOD	AS
RSE (EU/ml)	0.040	0.100
R-848 (μg/ml)	0.050	0.150
FSL-1 (ng/ml)	0.003	0.009

MVD and CLC Calculation

- K= 5 EU/kg (as for any parenteral administration);
- M = dose (ml)/body mass (kg) where dose = 0,25 ml and body mass= 5 kg (since it is a pediatric vaccine);
- LOD (single PBMC donor) = 0,04 EU/ml
- Assay sensitivity [AS] = 0,1 EU/ml (the lowest or one of the lower concentration of RSE or NEPs close to the beginning of the linear part of the standard curve)

CLC = K/M = 100 EU/ml
MVD* = CLC/LOD = **2700**
MVD° = CLC/AS= **1000**

Etna MP et al., **ALTEX 37(4), 532-544**. doi:10.14573/altex.2002252

* As described in European Pharmacopoeia

° Proposed new calculation

Layout of MAT applied to the pyrogenicity testing of TBEV vaccine

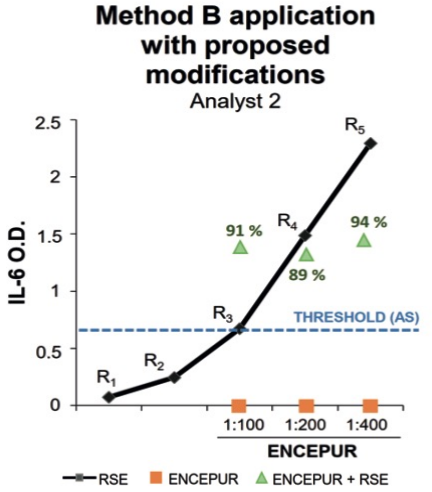
Active substance: TBEV inactivated by formaldehyde [ENCEPUR®]
Excipients: Aluminum hydroxide, TRIS buffer, sucrose.
Traces of tetracycline, gentamicine, neomycine and formaldehyde.

Method: Semi-quantitative (modified respect to Ph. Eur. v10.0)
Cell source: cryopreserved human PBMCs
Read-out: IL-6 release

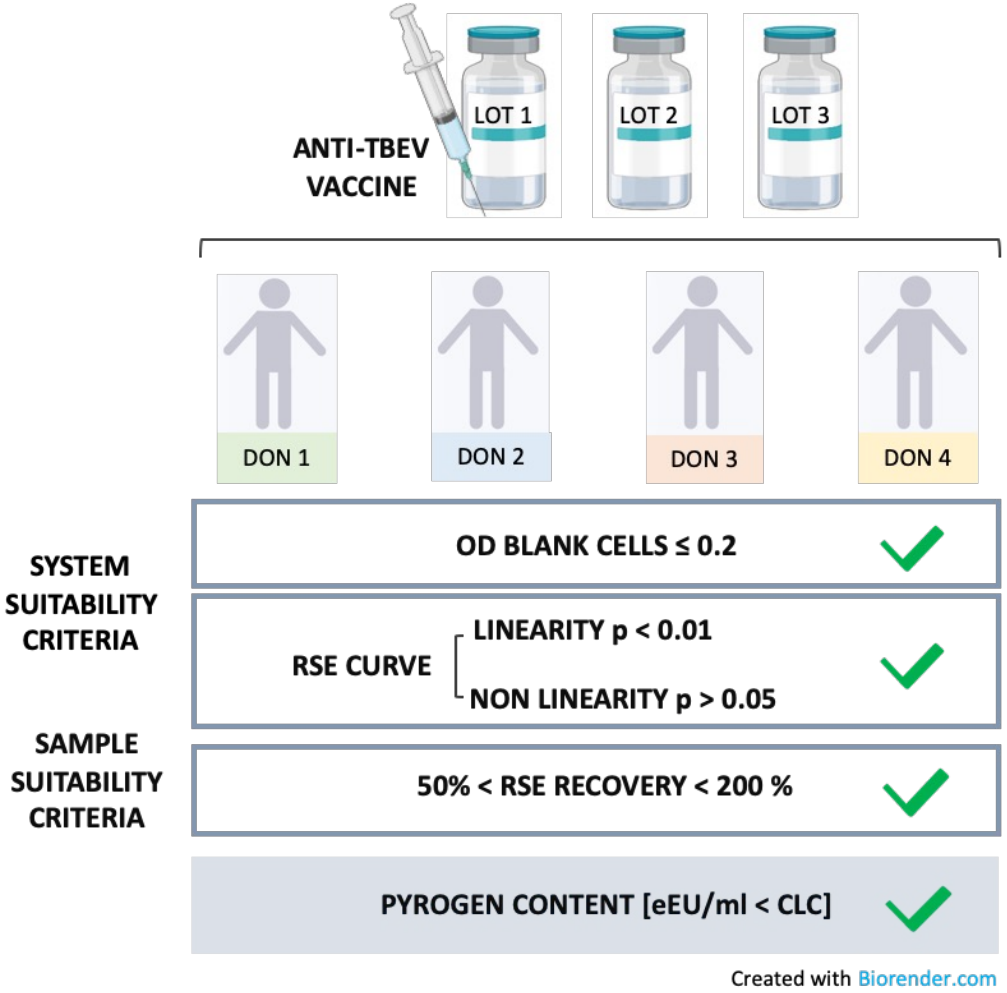
V1, V2, V3: Defined vaccine serial dilution;
E1, E2, E3, E4, E5: RSE chosen serial dilutions showing a linear correlation.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CELL Blank	Lot1 V1	Lot1 V2	Lot1 V3	E1	E2						
B												
C												
D												
E	ELISA Blank	Lot1 V1+E2	Lot1 V2+E2	Lot1 V3+E2	E3	E4	E5					
F												
G												
H	IL-6 Recomb											

Etna MP et al., **ALTEX 37(4), 532-544**. doi:10.14573/altex.2002252



MAT validation on 3 anti-TBEV vaccine lots



According to ICH Q2(R1), a precision study for the MAT optimized for the TBEV vaccine was conducted to calculate:

- **Repeatability** of the method
- **Intermediate precision** of the method

OD: Optical Density
RSE: Reference Standard Endotoxin
eEU/ml: Equivalent of Endotoxin Unit
CLC: Contaminant Limit Concentration

Thanks For Your Attention!

marilenapaola.etna@iss.it