10 YEARS OF BREAKTHROUGHS A HEALTHIER FUTURE

vac2vac

23

Application and optimization of Monocyte Activation Test for testing Tick Borne Encephalitis virus vaccine pirogenicity

Marilena P. Etna, Elena Giacomini, Patrizio Pezzotti, Christina Von Hunolstein, Eliana M. Coccia.

Facts & Figures

Start Date:	01/03/2016
End Date:	28/02/2021
Contributions	
IMI funding:	7 850 001 €
EFPIA in kind:	8 128 429 €
Other:	312 500 €
Total Cost:	16 290 930 €
Website:	www.vac2vac.eu

Challenge

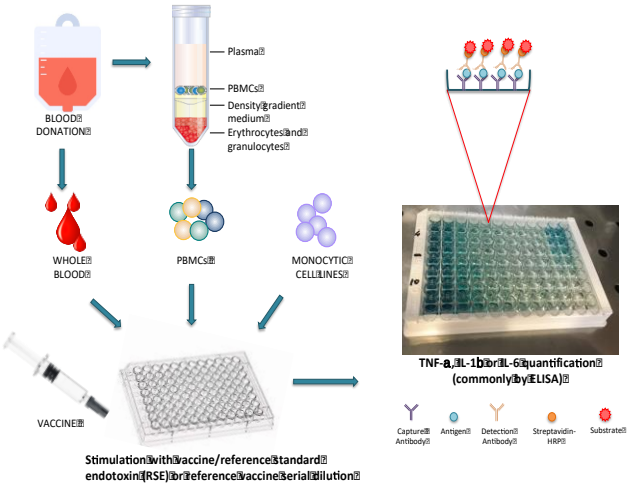
The overall objective of the "*Vaccine batch to Vaccine batch comparison by consistency testing*" project (VAC2VAC) is to develop and validate alternative, non-animal testing approaches for both human and veterinary vaccines providing data supporting the "consistency" of *in vitro* analytical based systems for quality control.

Accordingly, the high objective of our unit is to develop and optimize a cell based *in vitro* method for testing the pyrogen content of the human viral vaccine, i.e. the tick borne encephalitis virus (TBEV) vaccine, currently assessed *in vivo* through the rabbit pyrogen test (RPT).

Approach & Methodology

The MAT relies on the capacity of human monocytes or monocytic cells to sense substances able to stimulate the release of endogenous mediators of inflammation such as the pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) which have a role in fever pathogenesis. A scheme showing the cell isolation, vaccine stimulation and the cytokine determination by ELISA is inserted in **figure 1**.

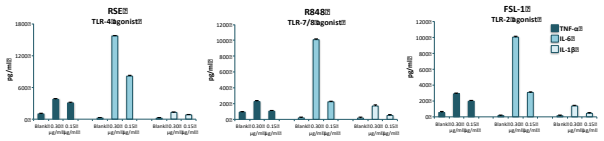
FIGURE 1. Monocyte activation test workflow.



Results

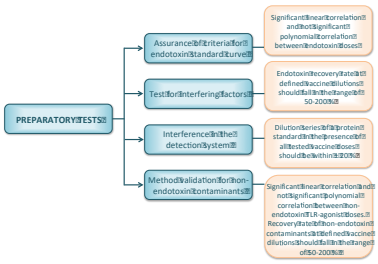
Among the possible cell type and read-outs of pyrogen-induced monocyte activation described in MAT monograph, human peripheral blood mononuclear cells (h-PBMC) were chosen as cell source for their high sensitivity and IL-6 as pyrogen-induced cytokine given its robust production from h-PBMC stimulated with a reference standard endotoxin (RSE), R-848 or FSL-1 (as non-endotoxin contaminants)(**Figure 2**).

FIGURE 2. Cytokine release after h-PBMC stimulation with endotoxin and non-endotoxin pyrogens.



To ensure both the precision and the validity of the assay and to allow the product-specific validation of MAT for the TBEV vaccine, the preparatory tests showed in figure 3 together with their acceptance criteria, have been conducted.

FIGURE 3. Preparatory tests for MAT assay optimization.



Data of preparatory tests allowed the setting of the final plate layout for assessing TBEV vaccine pyrogen content (**Figure 4**). In particular, three dilutions, in quadruplicate, of three different vaccine batches can be examined in a single experimental session.

FIGURE 4. MAT assay plate layout optimized for the TBEV vaccine.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank (cells only)	TBEV vaccine lot 1 1:100	TBEV vaccine lot 1 1:200	TBEV vaccine lot 1 1:400	TBEV vaccine lot 2 1:100	TBEV vaccine lot 2 1:200	TBEV vaccine lot 2 1:400	TBEV vaccine lot 3 1:100	TBEV vaccine lot 3 1:200	TBEV vaccine lot 3 1:400	RSE 0.8 EU/ml	RSE 0.4 EU/ml
B												
C												
D												
E	LAL Standard 600 pg/ml	TBEV vaccine lot 1 1:100 + RSE	TBEV vaccine lot 1 1:200 + RSE	TBEV vaccine lot 1 1:400 + RSE	TBEV vaccine lot 2 1:100 + RSE	TBEV vaccine lot 2 1:200 + RSE	TBEV vaccine lot 2 1:400 + RSE	TBEV vaccine lot 3 1:100 + RSE	TBEV vaccine lot 3 1:200 + RSE	TBEV vaccine lot 3 1:400 + RSE	RSE 0.2 EU/ml	RSE 0.1 EU/ml
F												
G	ELISA Blank											
H												

Value of IMI collaboration

The adaptation of MAT for testing the TBEV vaccine would not be possible without the opportunity to discuss our results in VAC2VAC consortium with both pharmaceutical partners and regulators in order to speed up the achievement of our goal.

Impact & take home message

The high sensitivity and reproducibility of the MAT optimized for the viral vaccine against TBEV will broaden the application of this test to other viral vaccines, likely possessing low- or no-intrinsic pyrogenicity, thus limiting the use of the RPT in quality or manufacturing process controls. Importantly, the MAT, as a replacement of the RPT, is in line with the application of the 3Rs for the replacement, reduction and refinement of animal use whose international harmonization is strongly promoted by European scientific community.

imi innovative medicines initiative efpia

This VAC2VAC project has received funding from the Innovative Medicine Initiative 2 Joint Undertaking under agreement N° 115924. This Joint Undertaking receives support from European Union Horizon's 2020 research programme and EPFIA.