MylcMAT™, pyrogen-detection system using immortalized human monocyte cell line.

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Introduction

Monocyte-activation test (MAT) utilizes human peripheral blood mononuclear cells (PBMCs) and/or monocyte cell lines for the detection of pyrogens. We have the platform to establish immortalized human monocyte cell lines, aMylc or iMylc, from PBMCs or iPS cells, respectively. We have previously reported a novel MAT using aMylc cell line, MylcMAT™ (ver.1) consisting of 20-hr cell-culture and followed by IL-6 measurement using ELISA (taking 5-hr).

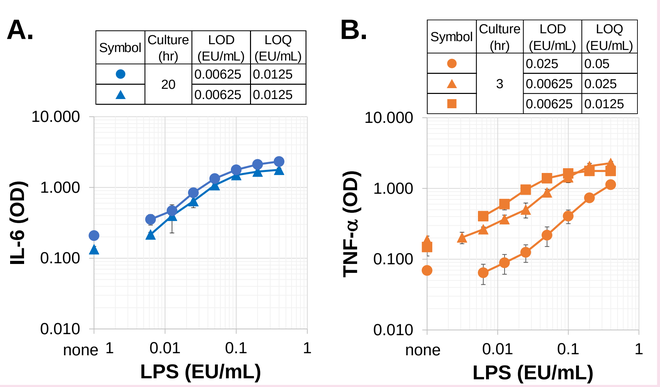
Purpose

Here we tried to develop MylcMAT™ (ver.2) comprised of 3-hr cell-culture and 1-hr TNF-α-detection.

MylcMAT™	Time (hr)		Target
	aMylc + sample	Detection of cytokine	
ver. 1	20	5 (ELISA)	IL-6
ver. 2	3	1 (Lumit™)	TNFα

Methods

Freshly thawed aMylc cells were cultured with LPS or several non-endotoxin pyrogens (NEPs). The amount of TNF-α or IL-6 in 3-hr or 20-hr culture supernatants (SNs), respectively, was measured by ELISA and/or Lumit™ (Promega).



Symbol	Culture (hr)	LOD (EU/mL)	LOQ (EU/mL)
●	20	0.00625	0.0125
▲		0.00625	0.0125

Symbol	Culture (hr)	LOD (EU/mL)	LOQ (EU/mL)
●	3	0.025	0.05
▲		0.00625	0.025
■		0.00625	0.0125

Figure 1

aMylc cells just after thawing are usable in drawing a standard curve for both IL-6 and TNF-α.

(A) aMylc cells were cultured with a titrated amount of LPS for 20-hr and the amount of IL-6 in SNs was measured by ELISA. The insert shows LOD and LOQ for individual experiments. **(B)** aMylc cells were cultured as in (A), and TNF-α in 3-hr SNs was measured by ELISA. Individual experiments are shown, represented by different symbols. n= 3 or 4 per group.

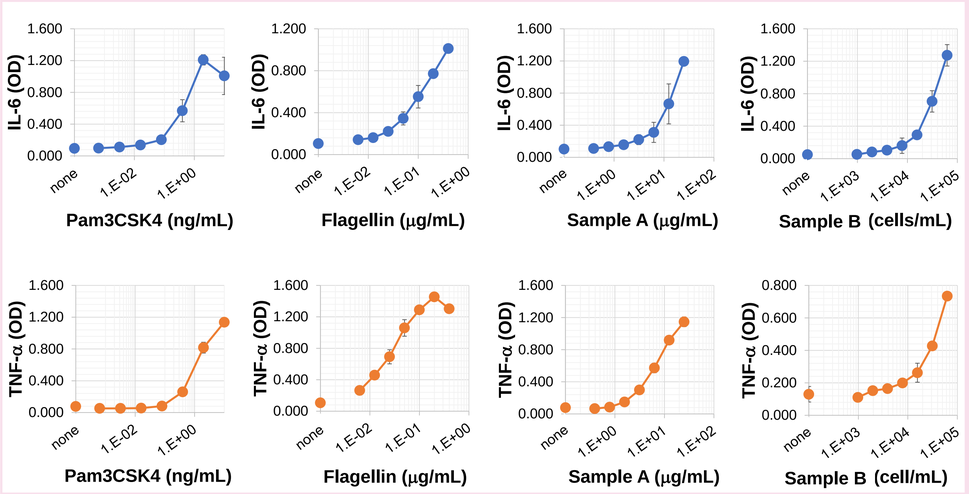


Figure 2

IL-6 and TNF-α are produced in a similar trend, in a dose-dependent manner.

aMylc cells were cultured with a titrated amount of NEPs or samples indicated. IL-6 in 20-hr culture SNs **(top panels)** and TNF-α in 3-hr culture SNs **(bottom panels)** were measured by ELISA. Each figure is a result from different experiment. N= 3 or 4 per group.

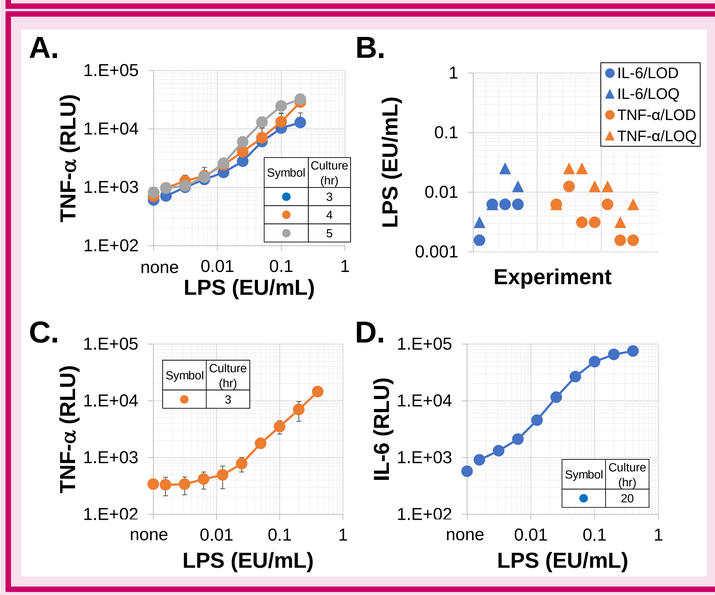


Figure 4

The sequential measurement of TNF-α and IL-6 from the same culture wells is possible by using Lumit™.

(A) aMylc cells were cultured with LPS at the indicated dose in a total 200 μL/96-well plate. At the indicated culture-period, 10 μL of SN from each well was harvested and used for TNF-α measurement. Lumit™ was used for the detection of cytokines instead of ELISA. The amount of cytokines is shown in RLU (relative light unit). **(B)** Based on the measurement of IL-6 (19~21-hr SN) and TNF-α (3~5-hr SN) by Lumit™ in an LPS-dose response, LOD and LOQ was calculated and summarized. Symbols on the same y-axis are derived from the same experiment. **(C)** aMylc cells were cultured as in (A). Three hours later, 10 μL of SN from each well was used for TNF-α measurement. The remaining wells (190 μL/w) were cultured further, and **(D)** total 20-hrs later, 10 μL of SN from each well was used for IL-6 measurement.

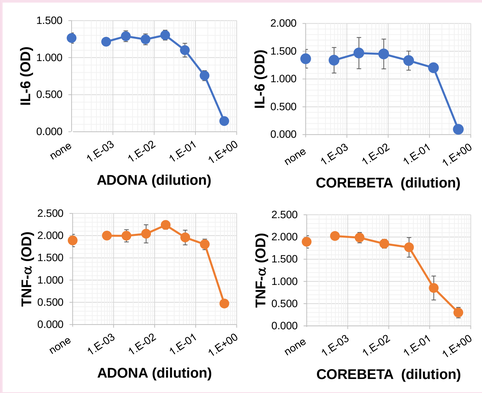


Figure 3

The production of IL-6 and TNF-α in response to LPS in the presence of medical samples is also in the same tendency.

Based on EP2.6.30 method A, aMylc cells were cultured with a titrated amount of two medicines spiked with a constant dose of LPS (0.2 EU/mL final). IL-6 in 20-hr culture SNs **(top panels)** and TNF-α in 3-hr culture SNs **(bottom panels)** were measured by ELISA. Each figure is a result from different experiments. n= 3 or 4 per group.

Conclusions

- MylcMAT™ is able to detect pyrogens in a total 4-hr assay (MylcMAT™ (ver.2): 3-hr culture + 1-hr TNFα measurement), in addition to a total 2-days assay (MylcMAT™ (ver.1): 20-hr culture + 5-hr IL-6 measurement).
- Furthermore, a sequential measurement of double parameters (TNF-α and IL-6) for pyrogen detection in the sample of interest is also possible, leading to more reliable results.