The Monocyte Activation Test Permits Testing of Medical Products and Devices While Detecting a Broad Range of Pyrogens

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INTRODUCTION: All manufactured substances, parenterally administered, soluble, or particulate, must undergo testing for microbial contaminations that may endanger the recipient patients. Over the past decades, the injection and implantation of rabbits has been the gold standard to identify such contaminations. However, this *in vivo* testing has received both regulatory and public scrutiny due to the animal cruelty it involves. In Europe, the rabbit testing already has been banned and its replacement is expected worldwide. This transition is enabled by the availability of *in vitro* testing systems that detect contaminations with equal or better sensitivity than the rabbit pyrogen test (RPT). In particular, the limulus amebocyte lysate (LAL) assay has emerged as a sensitive method for detecting lipopolysaccharide (LPS) contaminations. It's shortcoming however, is that it fails to identify contaminants other than lipopolysaccharide (LPS) also called endotoxins. Presently the monocyte activation test (MAT) is the forerunner for *in vitro* testing because it holds the potential to detect a broad range of contaminants that evoque systemic reactions in humans, other than endotoxin. In the MAT the very cells that respond in the human body to the contaminants, the monocytes, are exposed to test substances *in vitro* and the very response of cells to such exposure causing the fever reaction is being measured, namely the release of IL-6. One of the primary scopes of this work has been to establish that the MAT indeed detects a broad range of contaminants in addition to LPS. The second scope was to test whether the MAT is suited for the detection of pyrogens on the surface of medical devices as these contaminants are frequently not soluble, and thus cannot be detected by rinsing the device, yet *in vivo* they may cause adverse reactions. The LAL and RPT are only suitable to test rinsing solutions. The ability to detect pyrogens on medical devices is one of the last hurdles before the complete replacement of *in vivo* rabbit testing with an *in vitro* test system can be made.

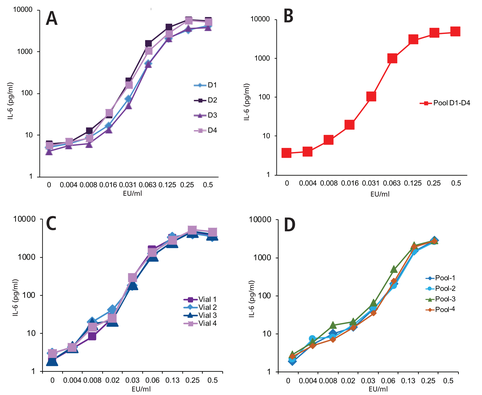

Figure 1: Detection of endotoxin-induced production of IL-6 in the MAT. LPS activates monocytes via toll-like receptor 4 (TLR4). (**A**) Peripheral blood mononuclear cells (PBMC) of four healthy human donors, specified by the symbols, were tested in the presence of the LPS concentrations specified on the X-axis. IL-6 was measured by ELISA in the culture supernatants after 20 hours of incubation. (**B**) Test results obtained with the PBMC of the four donors pooled in equal ratios. The MAT was performed as described in **A**. Note the low IL-6 background in the absence of LPS showing that mixed lymphocyte reactions within the 20 hour assay duration do not induce IL-6 production when the PBMC from four donors (with unmatched HLA types) are pooled. (**C**) Intra-batch reproducibility of the MAT. The pooled PBMC tested in **B** have been aliquoted in 700 cryovials, and cryopreserved. Four vials were thawed and were tested in the MAT as above. The data established that high inter assay reproducibility can be accomplished by the cryopreservation strategy. (**D**) Inter-batch reproducibility of the MAT. Four vials from four different batches of cryopreserved PBMC were thawed and tested as above. The data shows that comparable results with different batches of PBMC can be accomplished by our cryopreservation strategy.

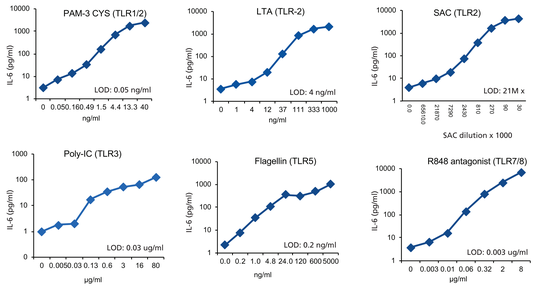

Figure 2: The MAT detects a broad range of non-endotoxin pyrogens. Six prototypic pyrogens, specified in the panels, have been tested in MAT. Next to the name of the pyrogen, the receptor is specified through which the activation of the monocytes occurs. The pyrogens have been plated into 96 well microtiter plates at the concentrations specified on the X-axis. PBMC were added and IL-6 was measured in the culture supernatants after 20 hours of incubation. The results are expressed as the IL-6 concentration detected by ELISA in the culture supernatant. The limit of detection (LOD) is specified for each TLR agonist. The data show that (unlike the LAL and the related Recombinant factor-C assays) MAT is suited to detect a wide range of microbial contaminants beyond LPS (a TLR4 agonist).

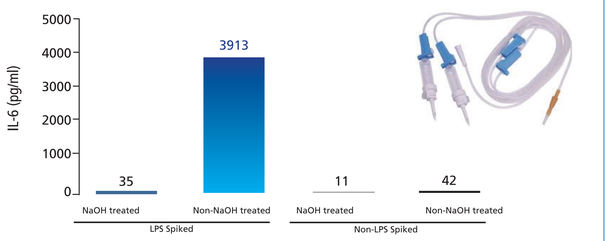

Figure 3: Testing solid medical devices by MAT, I. Transfusion set. The transfusion set shown has been infused with PBMC. After 20 hours, the liquid has been harvested, spun down, and IL-6 was measured by ELISA in the supernatant. This test was performed in four variations as specified underneath the bars. No significant IL-6 was observed in the non-spiked and non-NaOH treated infusion sets. In contrast, a very strong IL-6 signal was detected in the infusion set spiked with 0.333 EU/ml of LPS. The LPS recovery of 96% was calculated as the percentage of IL-6 detected after depositing of LPS in the device by spiking *versus* the IL-6 induced, by the same amount of LPS dissolved in media. This IL-6 signal was lost in the transfusion set that was NaOH treated consistent with the notion that NaOH inactivates LPS.

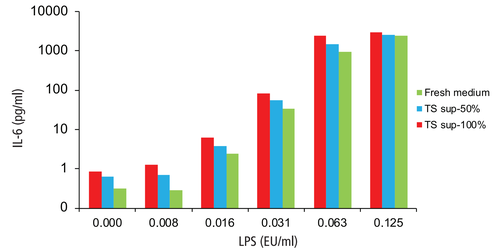

Figure 4: LPS neutralizing NaOH treatment has no toxic effect on PBMC leaving MAT results unaltered. This experiment was performed in order to find out whether NaOH treatment has any toxic effect on PBMC viability. An LPS standard curve was generated by diluting LPS in fresh medium (the green bars). Supernatants obtained from NaOH-treated transfusion sets were tested similarly, either as 100% supernatant (red bars) or as 50% supernatants with 50% fresh culture medium added (blue bars).

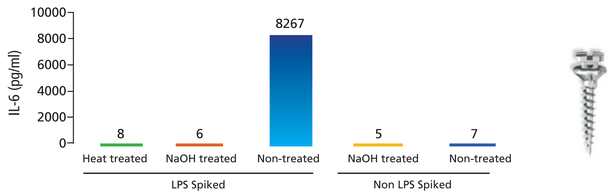

Figure 5: Testing solid medical devices by MAT, II. Spider screw. The spider screw used in orthodontic applications shown in the figure was tested under the conditions specified. The screw was submerged in a 2 ml tube containing 1.5 ml of culture medium with 2x10^5 cells/ml (pooled PBMC) and incubated at 37°C, 5% CO_2 for 20 hours. After centrifugation,the supernatant was tested in an IL-6 ELISA.. Before testing the screw was either spiked, or not with 0.333 EU/ml LPS. The spiked, and not spiked devices tested with or without additional NaOH treatment, as specified. LPS recovery was 108%, as specified in Figure 3. The LPS spiked screws have also been heat treated by exposure to 275°C for 6 hours.

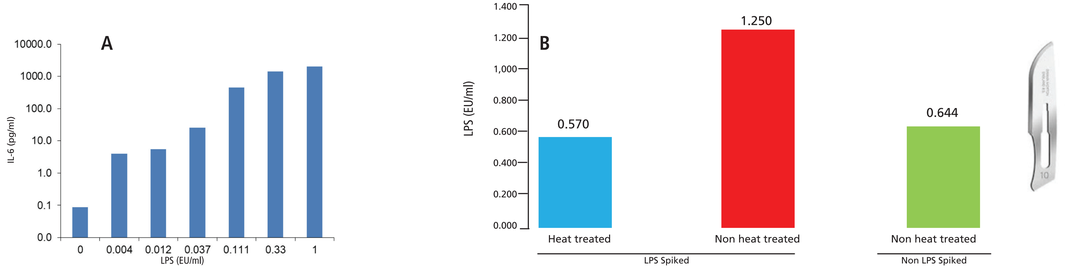

Figure 6: Heat treatment neutralizes LPS thereby distinguishing between contamination and material mediated pyrogenicity. A surgical scalpel blade was spiked with 0.333 EU/ml LPS and tested for IL-6 production in the MAT (red bar). The detected IL-6 was normalized to the LPS standard curve shown in A. Blades similarly spiked have been tested after dry heat treatment (blue bar) show in B. The results obtained with untreated blades are represented by the green bar. The surgical scalpel blade has been heat treated by exposure to 275°C for 6 hours.

CONCLUSIONS:
1. The cryopreservation of PBMC in large batches is well suited to be used as a reproducible source of monocytes for the MAT. **2.** High reproducibility can be accomplished by pooling PBMC of individual donors. **3.** The MAT is suited for detecting a wide range of non-endotoxin pyrogens, in addition to endotoxin. **4.**The MAT can be adapted for testing solid medical devices. **5.** The extent of LPS contamination detected after spiking corresponds to the amount of LPS deposited. **6.** Depyrogenation permits to verify a pyrogen induced effect *versus* material toxicity.