Scientific Confidence in the Monocyte Activation Test: A Human-Relevant Pyrogenicity Test for Medical Devices

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Abstract/Poster
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Background

- Pyrogenicity, the ability of a substance to induce fever, is a critical safety consideration in the evaluation of medical devices.
- This response is mediated by immune recognition of foreign substances, leading to cytokine release and hypothalamic modulation of body temperature.
- Pyrogens are broadly classified as either endotoxins, which are primarily lipopolysaccharides (LPS) from gram-negative bacteria, and non-endotoxin pyrogens (NEPs), which include a diverse array of biological and chemical agents.
- Historically, the rabbit pyrogen test (RPT) has been the standard assay for detecting pyrogens; however, regulatory agencies are encouraging transition to the use of more human-relevant and quantitative methods.
- The monocyte activation test (MAT) is a quantitative *in vitro* assay that uses human monocytes or monocytic cells to measure cytokine release, providing a mechanistically relevant approach for pyrogen detection.[1] As such, there is interest in conducting case studies to further demonstrate that the MAT can detect both endotoxin and NEPs in medical device contexts.
- Additionally, there is a growing scientific consensus that material mediated pyrogens (MMPs; listed in Annex G of ISO 10993-11:2017) are not relevant to the pyrogenicity testing of medical devices.[2]

Methods

- Following discussion at a 2018 workshop[3], we evaluated six types of endosseous dental implants ("spider screws") to assess the MAT's ability to detect both endotoxins and NEPs on device surfaces within this narrow context of use. (Table 1)
- These samples were spiked with known pyrogens (LPS, HKSA, R848).
- Devices were tested with and without dry heat, multiple spike controls, and dilution series (LPS/HKSA/R848 dose–responses) to assess spike recovery and to identify potential contamination.
- Additionally, seven MMPs listed in ISO 10993-11:2017 Annex G were tested using the MAT to determine their potential to elicit a pyrogenic response. (Table 2)
- Testing was conducted by MAT BioTech using pooled peripheral blood mononuclear cells (PBMCs) from four donors. Cytokine release was quantified via enzyme-linked immunosorbent assay (ELISA), using an IL-6 standard to generate a four-parameter logistic curve. (Figure 1)
- After data analysis, the scientific confidence framework outlined in the 2024 Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) report, "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies," was used to evaluate the MAT for detecting surface pyrogens on medical devices.

Type of Endosseous Dental Implant	Name	510(k) number [FDA product code]
1	Dentos AbsoAnchor® Orthodontic Microimplant SH 1514-08	K082838 [DZE]
2	Lancer Storm™ Mini Screw SAMS12	K062733 [OAT]
3	PSM Medical Quattro® MINI Screw ST-33-15007	K060126 [DZE]
4	Osstem OrthAnchor Mini Screw OSTH1608	K110392 [OAT]
5	ORLUS Mini Screw 1O16107	K000643 [DZE]
6	tomas®-pin SD 08 302-108-00	K042965 [OAT]

Table 1: List of endosseous dental implants evaluated.

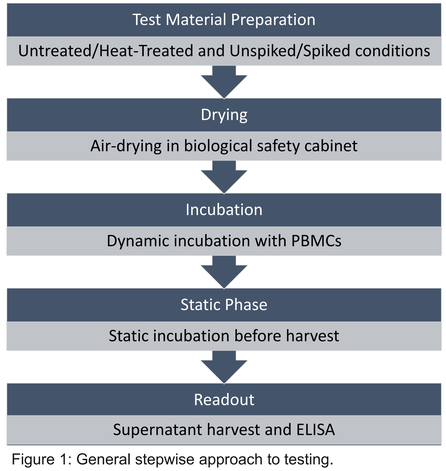

Figure 1: General stepwise approach to testing.

Results

- In all spiked conditions (LPS, HKSA, R848), heat-treatment successfully eliminated the pyrogenic signal, reducing IL-6 release to background levels comparable to negative controls. (Figure 2)
- Most of the screws tested showed acceptable spike recovery ranges between 50%-200% (Ph. Eur[4]) with the exceptions of type 1 HKSA, type 2 HKSA, and type 3 LPS. (Table 2)
- Types 2-6 used an optimized incubation matrix volume of 4 mL after it was discovered that the original volume of 2 mL was found to cause matrix-dependent interference.
- Type 1 was not optimized due to time constraints of the study.
- Type 2 demonstrated IL-6 response in untreated conditions, suggesting contamination of unknown origin that would have likely been mitigated by heat-treatment.
- Type 3 LPS recovery was very near the acceptable range and requires further investigation.
- None of the seven MMPs listed in ISO 10993-11:2017 Annex G elicited a pyrogenic response in the MAT. (Table 3)

Batch Name	LPS Recovery (%)	HKSA Recovery (%)	R848 Recovery (%)	Matrix Volume
Type 1 (AbsoAnchor)	79	387	79	2 mL
Type 2 (Storm Mini)	89	215	59	Optimized to 4 mL
Type 3 (Quattro)	47	123	119	
Type 4 (OrthoAnchor)	64	81	95	
Type 5 (ORLUS)	64	58	95	
Type 6 (tomas-pin)	63	63	93	

Table 2: Spike recovery percentages in cell culture matrix volume.

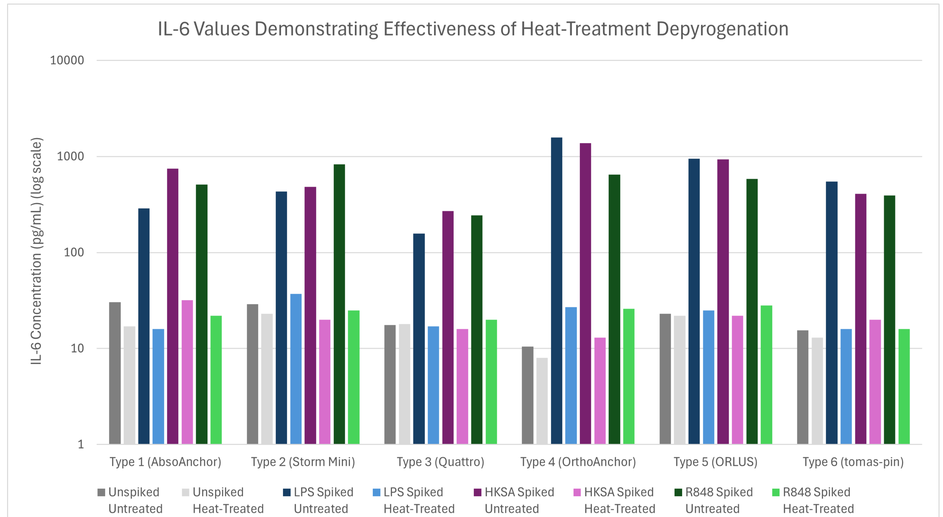

Figure 2: Effectiveness of heat-treatment as measured by IL-6.

MMP Name	MMP Alone (IL-6 Signal)	MMP + HKSA (IL-6 Signal)	Interpretation
N-phenyl-2-naphthylamine	(-) Negative	(-) Negative	Cytotoxic/Inhibitory
Picric acid (TNP)	(-) Negative	(+) Positive	Non-Pyrogenic
2,4-dinitrophenol (2,4-DNP)	(-) Negative	(+) Positive	Non-Pyrogenic
Prostaglandin E^2 (PE2)	(-) Negative	(+) Positive	Non-Pyrogenic
Polyinosinic–polycytidylic acid [Poly(I:C)]	(-) Negative	(+) Positive	Non-Pyrogenic
1-naphthylamine (1-NA)	(-) Negative	(-) Negative	Cytotoxic/Inhibitory
Nickel (II) phthalocyanine-tetrasulfonic acid (NiPcTS)	(-) Negative	(+) Positive	Non-Pyrogenic

Table 3: ISO 10993-11:2017 Annex G MMPs evaluated.

Conclusions

- Based on the study findings, the MAT is effective for detecting surface pyrogens on endosseous dental implants.
- All six screws will be retested using optimized matrix volume and standardized sample preparation to improve recovery comparability.
- The absence of pyrogenic response from the seven tested MMPs further supports the growing consensus that these substances are not relevant to pyrogenicity testing.
- Evaluated through the 2024 ICCVAM scientific confidence framework[5], the MAT demonstrates human biological relevance and reproducibility for this context of use.
- These findings support the MAT's role as a fit-for-purpose NAM for ensuring the safety of medical devices, shifting toward more ethical, accurate, and mechanistically sound approaches in medical device pyrogenicity testing.

References

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